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Review article

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Modelling and targeting mechanical forces in organ fibrosis

Shamik Mascharak[1,2,3], Jason L. Guo[1,2,3], Michelle Griffin[1,2,3], Charlotte E. Berry[1], Derrick C. Wan[1] ✉ & Michael T. Longaker[1,2] ✉

Abstract

Few efficacious therapies exist for the treatment of fibrotic diseases, such as skin scarring, liver cirrhosis and pulmonary fibrosis, which is related to our limited understanding of the fundamental causes and mechanisms of fibrosis. Mechanical forces from cell–matrix interactions, cell–cell contact, fluid flow and other physical stimuli may play a central role in the initiation and propagation of fibrosis. In this Review, we highlight the mechanotransduction mechanisms by which various sources of physical force drive fibrotic disease processes, with an emphasis on central pathways that may be therapeutically targeted to prevent and reverse fibrosis. We then discuss engineered models of mechanotransduction in fibrosis, as well as molecular and biomaterials-based therapeutic approaches for limiting fibrosis and promoting regenerative healing phenotypes in various organs. Finally, we discuss challenges within fibrosis research that remain to be addressed and that may greatly benefit from next-generation bioengineered model systems.

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Outlook

[1]Department of Surgery, Division of Plastic and Reconstructive Surgery, Stanford University School of Medicine, Stanford, CA, USA. [2]Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA, USA. [3]These authors contributed equally: Shamik Mascharak, Jason L. Guo, Michelle Griffin. ✉e-mail: dwan@stanford.edu; longaker@stanford.edu

Key points

- Fibrosis — the replacement of normal tissues with matrix-rich connective tissue — is a common response to trauma, autoimmune reactions, radiation and other insults, and can occur in almost every organ.
- Fibrotic disease is initiated and propagated by a variety of physical forces, including cell–matrix, cell–cell, osmotic and viscous, as well as physical confinement and nuclear forces.
- Fibrosis models can be engineered to study mechanotransduction pathways specific to each type of physical force.
- Molecular and biomaterials-based therapies have been developed to treat fibrosis by modulating mechanotransduction.
- Multiomics analysis, next-generation models and biomaterials as well as advanced tools for the quantification of fibrosis will further advance fibrosis research.

Introduction

Fibrosis is the replacement of healthy tissue with extracellular-matrix (ECM)-rich connective tissues produced by fibroblasts and other stromal cells, occurring in almost every organ in the body after injury. In the acute context (for example trauma), such 'scarring' processes are indispensable for rapidly restoring organ function and are at least partially reversible; thus, the disruption of normal parenchymal architecture is minimized. For example, scars, contractures and keloids are acute fibroses that can occur after skin injury (such as laceration, burn and surgeries), with over 80 million scars and keloids estimated to occur annually in high-income countries, which is related to healthcare expenditures exceeding US$15 billion[1–3] (Box 1). Chronic, repetitive or large-scale injuries (autoimmune inflammation, long-term infection, radiation, drugs) can cause excessive deposition and minimal breakdown of matrix proteins, resulting in permanent parenchymal distortion and fibrotic organ dysfunction (such as liver cirrhosis, pulmonary fibrosis or scleroderma). Importantly, fibrosis may cause subsequent diseases (for example heart failure following myocardial infarction), hospitalization and psychosocial distress[4]. Across all organs, fibrosis is involved in 45% of deaths in high-income countries[5]. However, only a few efficacious therapeutic options exist for fibrotic diseases (for example nintedanib and pirfenidone for idiopathic pulmonary fibrosis), and most therapies cannot prevent long-term disease progression, which is also related to the limited knowledge of the fundamental causes of organ fibrosis.

Innate and adaptive immune responses, infectious organisms (such as viruses or helminths) and genetic factors (for example telomerase mutations in idiopathic pulmonary fibrosis) have been implicated as important drivers of fibrosis[6,7]. Mechanical forces, including stretch, shear and deformation, that cells experience in the profibrotic niche (defined here as the various inflammatory cytokines, activated cells, dysregulated ECM and other stimuli within the injury microenvironment) may also play an important role in the onset and progression of fibrosis[8]. In addition, fibrotic tissues are often stiffer than their healthy counterparts[8]; for example, a nodular cirrhotic liver can be palpated and diagnosed through a physical exam as it is stiffer than a healthy liver. The specific mechanisms by which mechanical forces initiate and propagate pathological fibrosis are being increasingly elucidated and may also be targeted by treatments.

In this Review, we discuss the molecular and cellular mechanisms by which different sources of mechanical force contribute to fibrotic diseases of the skin, lungs, heart and other organs. We highlight in vitro, in silico and in vivo model systems that can be applied to investigate fibrotic signalling pathways, as well as translational efforts to prevent or reverse existing fibrosis. We conclude with a survey of future directions and unmet needs within fibrosis research, for which next-generation bioengineered approaches will be necessary.

Mechanotransduction mechanisms in fibrosis

Mechanotransduction refers to the mechanisms by which cells convert physical stimuli (for example cell–matrix forces, cell–cell forces, osmotic and interstitial fluid pressure shifts, fluid shear stress, and physical confinement and compression) into discrete changes in cell signalling and behaviour (for example proliferation, migration, changes in gene expression, differentiation; Fig. 1)[9–14]. Mechanoresponsive cells sense physical stimuli through a diverse array of extracellular, membrane-bound, cytosolic and nuclear factors.

Cell–matrix forces

Reciprocal interactions between cells and the surrounding ECM through transmembrane proteins are a key feature of fibrotic diseases across multiple organs. For example, integrins bind and cluster around specific amino acid sequences (including RGD, YIGSR and DGEA) within ECM proteins (such as fibronectin, laminin, collagen and elastin), complexing intracellularly with an array of linker proteins, such as talin, vinculin and paxillin[15]. The resulting focal adhesions serve as anchor points that link the ECM to the cell membrane and actomyosin cytoskeleton, which, in turn, generates contractile forces that reveal new binding sites on ECM proteins[16]. This positive feedback loop 'matures' the focal adhesion and reorganizes the actin cytoskeleton to promote cellular adhesion and migration. Focal adhesions also harbour signalling complexes, such as focal adhesion kinase (FAK), leading to the activation of various downstream mediators, including mammalian target of rapamycin (mTOR) (promoting protein synthesis and growth), Rho-associated protein kinase (Rho/ROCK) (triggering cytoskeletal reorganization and proinflammatory cytokine expression) and mitogen-activated protein kinase (MAPK) (various effects on differentiation and proliferation)[17,18]. Through this process of maturation, cytoskeletal reorganization and activation of downstream secondary messengers, the focal adhesion serves as a key mechanosensing apparatus for the cell.

Fibroblasts are particularly sensitive to shifts in cell–matrix mechanical forces, exhibiting increased migration and proliferation, as well as upregulated expression of matrix proteins, growth factors such as transforming growth factor (TGF)-β, and inflammatory cytokines in response to increasing substrate stiffness, cyclic stretch or microdeformation by suction[19–21]. For example, using micromechanical loading devices in a mouse model of wound healing, it was shown that increased tension activates FAK, which, in turn, promotes secretion of monocyte chemoattractant protein 1 (MCP1) and interleukin (IL)-4 and IL-13 to increase recruitment of macrophages and T cells[22,23]. Similarly, wound tensile forces (0.15–0.27 N mm^{-2}, generated by mechanical distraction devices affixed to the dorsal skin) trigger postnatal activation of the homeobox protein Engrailed-1 (En1) in previously En1-naive mouse fibroblasts of the lower dermis (Engrailed-1 lineage-negative fibroblasts (ENFs); experiments performed in tamoxifen-inducible En1 transgenic

reporter mice), a phenomenon dependent on Yes-associated protein (YAP) that generates a new 'scar lineage' (Engrailed-1 lineage-positive fibroblasts (EPFs)) crucial to wound fibrosis[23–25]. These mechanically activated fibroblast phenotypes may be considered part of the larger 'myofibroblast' phenotype, which refers to the common contractile (for example expressing alpha smooth muscle actin (α-SMA)) and adherent cell type responsible for fibrosis after injury across various organs.

In response to injury, such mechanotransduction mechanisms result in further ECM deposition, crosslinking and reorganization, which causes the formation of stiff fibrotic matrices, which trigger further mechanical activation. For example, fibrosis during skin wound healing (Box 1) results in the deposition of scar tissue rich in collagen type I, with an elastic modulus 2–3 times that of unwounded skin, driving further activation of the mechanotransduction signalling pathways highlighted above (for example FAK, YAP, TGFβ); thus, a feedforward loop is generated that amplifies tissue fibrosis[26]. Fibrotic lungs, which have elastic moduli up to 10 times as high as that of healthy lungs, are characteristically enriched for collagens type I, III and VI and deficient of laminins owing to disruption of the alveolar basal lamina[27,28]. ECM degradation occurs in tandem with ECM deposition and crosslinking during the acute injury response and for a prolonged period in the remodelling phase. In particular, matrix metalloproteinases (MMPs) are responsible for ECM degradation. MMPs are produced by various cells, including macrophages, and are tightly regulated by profibrogenic cytokines (for example TGFβ, epidermal growth factor (EGF) and IL-1), extracellular inhibitors (for example tissue inhibitors of MMPs) and post-translational modification[29]. In the lungs, changes in ECM composition collectively produce a rigid interstitial matrix that serves as a feedforward loop for additional profibrotic activation of alveolar type II cells and activation of lung fibroblasts into myofibroblasts[18].

Similar processes of ECM compositional change and connective tissue thickening have been implicated in the pathogenesis of intestinal fibrosis, including similar feedforward loops for myofibroblasts[30,31]. Cell–matrix engagement and YAP signalling in response to ECM compositional changes after injury also cause de-differentiation of colonocytes, hepatocytes and other epithelial cells into a 'fetal-like' state that is necessary for efficient epithelial regeneration[32,33]. Thus, YAP signalling is involved in both regenerative and fibrotic injury responses, depending on the cellular and organ context.

Beyond the activation of fibroblasts to myofibroblasts, which is a key contributing factor to scar formation, epithelial-to-mesenchymal transition (EMT) may also be involved in the fibrosis of multiple tissues[34]. For example, type II EMT, that is, the transdifferentiation of epithelial cells to myofibroblasts, has been implicated in scleroderma, atherosclerosis and idiopathic pulmonary fibrosis[34,35], and can be activated by YAP, Wnt/β-catenin (β-cat) and Notch signalling[35]. Interestingly, EMT may produce a spectrum of transitional phenotypes with varying epithelial and mesenchymal gene expression and distinct profibrotic transcriptional programmes, including production of proinflammatory cytokines (for example chemokine C–X–C motif ligand 2 (CXCL2), chemokine C–C motif ligand 3 (CCL3) and IL-33) and cell cycle arrest[34]. Thus, EMT may contribute to fibrosis both by generating myofibroblasts and by establishing a profibrotic microenvironment. Cell–matrix mechanotransduction mechanisms are important regulators of EMT; for example, ECM engagement of α3-integrin can drive EMT in lung fibrosis by promoting β-catenin/SMAD signalling[36]. Interestingly, matrix degradation also seems to play a role in EMT, as processing of the laminin β1 chain by MMP2 was shown to generate a cryptic laminin fragment that interacts with α3-integrin and modulates expression of various EMT-associated genes (E-cadherin, MMP2 and MMP9)[37].

Box 1

Wound healing and fibrosis

The skin undergoes a stereotyped wound healing process following injury that inevitably results in scarring, a highly orchestrated fibrotic reaction causing the replacement of damaged tissues with an extracellular-matrix (ECM)-rich scar. Wound healing is typically divided into several phases of healing, beginning with haemostasis and the formation of a fibrin matrix. Within 2 to 10 days, wound repair enters a proliferative phase, characterized by angiogenesis, formation of granulation tissue, and reconstitution of the epithelial barrier by proliferation and migration of keratinocytes at the leading edge[252,253]. During this phase, fibroblasts also proliferate and migrate into the wound bed under the influence of growth factors and inflammatory cytokines, such as platelet-derived growth factor (PDGF) and transforming growth factor-β (TGFβ), differentiating into myofibroblasts that deposit ECM and contract the wound edges[253]. The final remodelling phase of wound healing is the longest phase, beginning roughly 2 weeks after injury and lasting months to years, as inflammatory cells undergo apoptosis, and immature scar matrix containing fibronectin and type III collagen is reorganized into mature matrix containing parallel, aligned bundles of type I collagen[253]. Despite extensive deposition and remodelling of matrix proteins, scar matrix is ultimately inferior to that of unwounded skin in terms of architecture and protein composition, and thus regains at most 75–80% of its original mechanical strength[254]. Scar matrix is also deficient of functional appendages, such as hair follicles and glands, and thus lacks the thermoregulatory control of unwounded skin.

Fibrosis outside wound healing can occur for multiple reasons and, in general, is not a response to a physical injury. It can occur over long timescales in response to persistent inflammation (autoimmune attack, infection), radiation, malignancy (desmoplasia) and chemical damage of skin and other organs. Fibrosis is typically not divided into the phases that apply to wound healing. Within such chronic contexts, long-term production of inflammatory and fibrogenic cytokines (such as TGFβ or tumour necrosis factor (TNF)), prolonged myofibroblast proliferation and matrix remodelling occur in tandem. As a result of imbalanced matrix deposition and breakdown, organs are gradually replaced by disorganized fibrotic scar tissue and ultimately fail.

Cell–cell forces

In addition to the forces produced by cellular interactions with the extracellular space, cells themselves can exert mechanical forces on their neighbours to modulate profibrotic activation; in particular, homotypic cell–cell interactions, mediated by the homodimerization of membrane-bound cadherins on neighbouring cells through adherens junctions[38]. For example, cadherin-11 co-expression promotes TGFβ signalling in myofibroblasts and macrophages during dermal fibrosis in mouse models, and cadherin-11 expression is elevated in the skin of patients with systemic sclerosis[39]. Moreover, in vitro atomic-force-microscopy

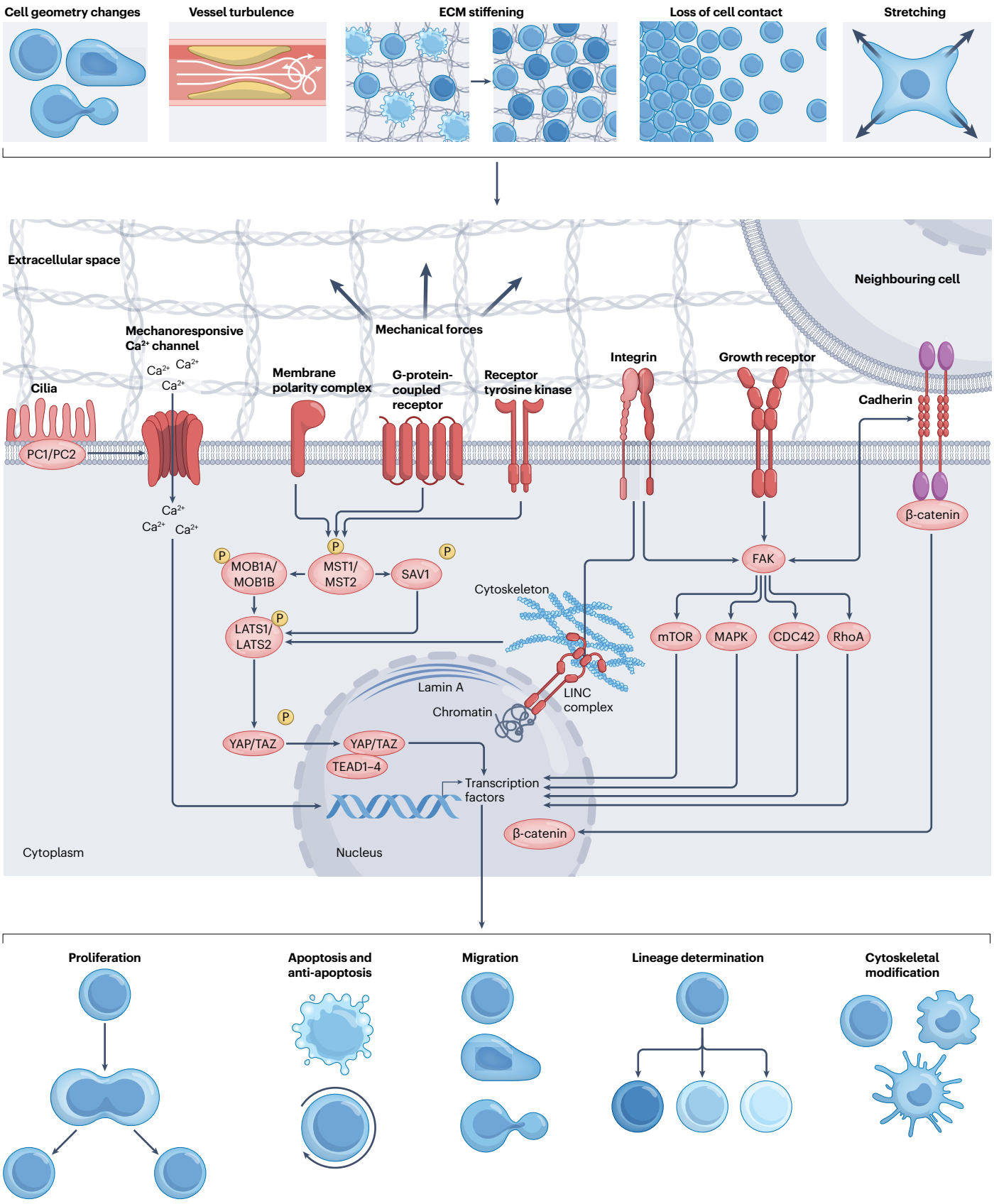
Cell geometry changes
Vessel turbulence
ECM stiffening
Loss of cell contact
Stretching
Extracellular space
Neighbouring cell
Mechanical forces
Mechanoresponsive Ca2+ channel
Ca2+
Cilia
PC1/PC2
Membrane polarity complex
G-protein-coupled receptor
Receptor tyrosine kinase
Integrin
Growth receptor
Cadherin
β-catenin
FAK
MOB1A/ MOB1B
MST1/ MST2
SAV1
LATS1/ LATS2
P
Cytoskeleton
mTOR
MAPK
CDC42
RhoA
Lamin A
Chromatin
LINC complex
YAP/TAZ
TEAD1–4
Transcription factors
Cytoplasm
Nucleus
Proliferation
Apoptosis and anti-apoptosis
Migration
Lineage determination
Cytoskeletal modification

Fig. 1 | Physical stimuli and associated downstream mechanotransduction signalling pathways. Physical perturbations, including geometric confinement, fluid shear stress, substrate stiffness, changes in cell–cell contact and osmotic forces, are sensed by an array of membrane and nucleus-associated receptors, including integrins, receptor tyrosine kinases, G-protein-coupled receptors, mechanoresponsive ion channels, cilia and membrane polarity complexes, causing intracellular signalling shifts through secondary messengers (for example Ca^{2+}, focal adhesion kinase (FAK) and Yes-associated protein (YAP)). These second messengers drive transcriptional changes through effects on various transcription factors (for example Yes-associated protein-TEA domain family member (YAP-TEAD), SMAD, nuclear factor κB subunit 1 (NFκB), β-catenin), leading to changes in cell proliferation, survival, differentiation and migration in the context of fibrotic disease. CDC42, cell division control protein 42 homolog; ECM, extracellular matrix; LATS1/2, large tumour suppressor kinase 1/2; LINC, linker of nucleoskeleton and cytoskeleton; MAPK, mitogen-activated protein kinase; MOB1A/B, Mob1 homolog 1A/B; MST1/2, macrophage stimulating 1/2; mTOR, mammalian target of rapamycin; PC1/2, polycystin 1/2; RhoA, Ras homolog family member A; SAV1, protein salvador homolog 1; TAZ, transcriptional coactivator with Pdz-binding motif.

experiments have demonstrated that cadherin–cadherin binding strength doubles in activated myofibroblasts[40].

Heterotypic cell–cell junctions can also transmit mechanical forces through different cadherin receptors. E-cadherin, for example, bridges hepatocytes to hepatic stellate cells (HSCs), which inhibits downstream YAP/TAZ signalling and profibrotic activation of HSCs[41]. Thus, disruption of adherens junctions between hepatocytes and HSCs may promote YAP/TAZ-mediated mechanotransduction pathways in HSCs[42]. Within these junctions, cadherins transmit cell–cell mechanical forces through complexes with cytoplasmic β-catenin, which further complexes with α-catenin and vinculin to link to the actin cytoskeleton[43]. Disruption of this complex by cell migration or exogenous mechanical force can directly promote the translocation of β-catenin into the nucleus, where it serves as a transcription factor for fibronectin, MMPs and other profibrotic agents in systemic sclerosis and other fibroses, both in vitro and in vivo[44,45].

In addition to disruption of adherens junctions, reorganization of the actin cytoskeleton around intact cell–cell junctions can inhibit large tumour suppressor kinases, causing disinhibition and nuclear translocation of YAP[17]. This, in turn, promotes proinflammatory (for example IL-1, IL-6 and MCP1) and profibrotic gene expression in a variety of organs[23,46]. Cytoskeletal reorganization may also cause the deformation of the lamin nuclear envelope or directly alter chromatin structure by sequestering histone deacetylases[47]. Therefore, transmission of cell–cell forces can promote profibrotic cell activation not only through the induction of actin cytoskeletal reorganization in a neighbouring cell, but also through the disruption of cell–cell contacts, resulting in the intracellular release of β-catenin.

Fluidic forces

Cells can respond to fluidic forces through cilia, microvilli, stretch-sensitive ion channels and other membrane-associated structures, which has important implications in homeostasis and fibrotic disease. For example, low levels of shear stress (the frictional force caused by fluid flow over cells), in the range of 0.1–0.5 dyn cm^{-2}, maintain endothelial, hepatocyte and HSC phenotype in the liver, including albumin, cytochrome P450 family protein and hepatocyte growth factor expression, as well as nitric oxide production in the endothelium[48]. However, following fibrotic disruption of the cirrhotic microvasculature, flow conditions show increased resistance, cyclic pressure and shear stress, ultimately resulting in endothelial cell dysfunction and innate immune cell activation through Notch-, integrin- and PIEZO1-mediated pathways. The subsequent expression of angiocrine factors by endothelial cells (for example CXCR4 and CXCR7) drives hepatocyte dysfunction and generation of myofibroblasts from HSCs[49,50].

Cell–cell junction proteins, such as platelet endothelial cell adhesion molecule (PECAM) and cadherins, are another important component of endothelial shear flow sensation, complexing with cytoskeletal components and various secondary messengers to modulate transcriptional regulators (Krüppel-like factor (KLF) 2/4, YAP, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), β-catenin) of NO synthesis, proliferation, migration and inflammatory cytokine production[51].

Epithelial cells, for example in the renal tubule, sense bending moments along primary cilia and apical microvilli in response to flow with shear forces in the 1–4 dyn cm^{-2} range[52]. These structures interact intracellularly with polycystins (PC), a family of signalling proteins that sense ciliary bending moments (PC1), promote Ca^{2+} influx (PC2), and interact with cell–cell junctions and cell–matrix proteins (PC1)[53,54]. Polycystins may additionally harbour binding pockets for ligands in the Hedgehog, epidermal growth factor receptor (EGFR), Wnt and TGFβ pathways[53]. Polycystins also influence the responsiveness of force-gated ion channels, such as transient receptor potential cation channel subfamily V member 4 and PIEZO1, which open upon deformation of the cell membrane under flow conditions to allow Ca^{2+} influx[55,56]. Calcium ions are thus shared secondary messengers in response to disrupted flow conditions, and influx of calcium may cause fibrosis through activation of extracellular-signal-regulated kinases (ERK) and Rho/ROCK, as well as promotion of EMT to generate fibroblast-like cells[57,58]. Interestingly, fibroblasts and respiratory epithelial cells show increased cilia formation in patients with idiopathic pulmonary fibrosis[59] and within reorganizing myocardial scars[60]; furthermore, fibroblast-specific PC1 deletion prevents cardiac remodelling after myocardial infarction in murine models[60]. Cilia thus seem to play an important functional role in fibrotic disease processes; however, their specific relationship with flow sensing in these conditions remains to be elucidated.

Cells also encounter compressive hydrostatic fluidic forces on macroscopic scales, as exemplified by cardiac hypertrophy in response to hypertension or valvular disease. Chronic pressure overload (20–40 mmHg, that is, tens of thousands of dyn cm^{-2}) in such conditions leads to diastolic dysfunction by stimulating cardiac myofibroblast activation and expression of α-SMA, collagen type I and TGFβ in a SMAD3 dependent manner[61,62]. In mouse models of chronic cardiac pressure overload, established by transverse aortic constriction, fibroblast activation of TGFβ/SMAD3 is protective to cardiomyocytes as it preserves the cardiac ECM network through suppression of proteases and macrophage-driven inflammation[63]. Interestingly, however, acutely increased hydrostatic pressures of 200 mmHg suppresses the cardiac myofibroblast phenotype through Akt phosphorylation and inhibition of glycogen synthase kinase 3 (GSK-3), thereby decreasing transcription of collagen types I and III[64]. The molecular mechanisms differentiating these divergent responses to compressive fluidic forces of varying magnitude and timescales remain to be investigated.

Osmotic and viscous forces

The extracellular fluid contains ions and macromolecules (such as mucins, glycosaminoglycans and serum proteins) that contribute to its density, viscosity and tendency to impose osmotic forces across cell membranes and cell–cell junctions. For example, defects in the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel cause increases in airway mucin granule viscosity and osmotic pressures across respiratory epithelial cells[65,66]. Using viscosity-enhancing polymer solutions, it has been shown that high viscosity of the extracellular fluid promotes integrin-dependent cell spreading and consequently actin cytoskeleton rearrangement, nuclear flattening and nuclear translocation of both YAP and β-catenin in vitro[67].

Osmotic fluid shifts across the membrane cause changes in membrane tension that are sensed by mechanoresponsive ion channels, such as TRP and PIEZO1, contributing to fibrosis through Ca^{2+} influx. Increased intracellular ionic strength then directly activates nuclear factor of activated T cells 5 (NFAT5), which translocates to the nucleus to affect transcription of genes involved in osmolyte transport (for example aquaporins), inflammation (for example *CCL2*, cyclooxygenase-2 (*COX2*)), and matrix production (for example collagen type I and aggrecan)[68]. Hyperosmotic conditions may also stimulate EMT, decreasing E-cadherin and increasing α-SMA expression in renal tubular epithelial cells through Rho-dependent rearrangement of focal adhesions[69]. Using Förster resonance energy transfer (FRET) force biosensors in CFTR-deficient Madin–Darby canine kidney (MDCK) cells, defects in chloride transport have been shown to increase internal hydrostatic pressure and circumferential stretch on adherens junctions in epithelial acinar structures[70]. Interestingly, increasing CFTR function and thereby offloading adherens junctions blocks TGFβ-induced EMT. Thus, the tendency of epithelial cells to undergo EMT and contribute to fibrosis in response to osmotic shifts may depend on a transfer of force away from cell–cell and towards cell–matrix adhesion.

Physical confinement and nuclear mechanotransduction

Physical confinement of cells in an ECM of variable collagen fibre density, porosity and spatial architecture leads to various degrees of compression that influence cell motility, morphology and cytoskeletal and nuclear deformation. For example, fibroblasts may migrate at differential rates in vitro depending on compressive confinement pressure[71]. Biaxial physical confinement can also inhibit cell cycle progression of sarcoma cells in models of fibrotic ECM, including microchannel devices coated with various extracellular ligands, such as collagen and fibronectin[72]. Nuclear deformation, however, is influenced by both physical confinement and cell migration, and nuclear components, such as lamin, chromatin and nucleoplasm, can deform as a result of forces transduced from the cytoskeleton[71].

Nuclear deformation can cause the modulation of gene expression in fibroblasts by exposing chromatin to mechanical cues[73,74]. This type of force transduction mainly occurs through the linker of nucleoskeleton and cytoskeleton (LINC) complex, a multiprotein complex that spans the inner and outer membranes of the nucleus, converting cytoskeletal force to downstream effects on genomic stability, DNA repair and more[74]. Notably, the LINC complex can physically bind to nuclear chromatin, modulating the degree of chromatin stretch and the resulting accessibility of transcription factor binding sites[74]. Accordingly, nuclear mechanotransduction via the LINC complex has been implicated in the epigenetic promotion of proinflammatory gene expression by fibroblasts in mouse models of cardiac fibrosis and other diseases[74–76]. Furthermore, the LINC complex can directly alter the permeability of nuclear pores to increase YAP/TAZ translocation, thereby promoting a profibrotic phenotype in fibroblasts and other cells[76,77]. Similarly, the LINC complex couples physical confinement and other mechanical perturbations to the nuclear influx of histone deacetylases (HDACs), which promote chromatin condensation and have been implicated in the pathogenesis of cardiac, liver, kidney and pulmonary fibrosis[78].

Mechanotransduction between the actin cytoskeleton and the nucleus is dependent on multiple proteins within the LINC complex, including Sad1 and UNC84 domain containing 1 (SUN1) and SUN2. These proteins are required for a multitude of profibrotic transcriptional programmes triggered at the LINC interface[74,79,80]. Disrupting cytoskeletal–nuclear mechanotransduction in mice using small-molecule inhibitors of actomyosin fibre contractility, such as blebbistatin, allows its mechanistic investigation; however, off-target effects related to cell contractility may orthogonally influence profibrotic behaviour[76]. Overall, nuclear deformation plays a crucial role in various profibrotic epigenetic processes, with the LINC complex serving as a major mediator of these adaptations at the nuclear membrane.

Models of the fibrotic mechanical environment

The development of therapeutics for the treatment of fibrosis has been limited by bottlenecks in the translation from basic science to clinical application[81,82]. The National Institutes of Health (NIH) estimate that approximately 0.1% of preclinical drug candidates are translated to federally approved drugs, and one major contributor to this low success rate is poor correlation between preclinical model efficacy and clinical efficacy in phase 2/3 trials[82]. Given the associated costs of US$2.6 billion on average for the full development process of an approved drug[82], this colloquially termed ‘valley of death’ in translational research represents a major factor to be addressed in the development of antifibrotic therapies.

Translational risk may be reduced by developing clinically representative models of fibrotic disease. Current in vivo and in vitro models of fibrosis do not fully recapitulate the complexity of disease progression in humans[81]. For example, small-animal models, such as mice, exhibit distinct mechanobiological factors during fibrosis compared with humans, including, but not limited to, rapid wound contraction in mice (which is not the case in humans), animal-specific differences in tensile and compressive behaviour for the same organ, and less variability in the kinetics of disease progression[83,84]. The recent passage of the Food and Drug Administration (FDA) Modernization Act 2.0 has authorized the usage of certain alternatives to animal models, such as cellular assays and computational models, to establish the efficacy and/or safety of some preclinical drug candidates[85]. The European Medicines Agency (EMA) also recently implemented similar measures to support animal model alternatives, including direct sponsorship by its Innovation Task Force to support pharmaceutical researchers in developing new approach methodologies to non-animal and in vitro drug testing[86]. Thus, antifibrotic drug development will benefit from the design of in vitro, in vivo, ex vivo and in silico model systems that more accurately recapitulate the complex spatiotemporal dynamics and mechanobiological signals characteristic of fibrotic disease progression, compared with animal models.

Modelling cell–matrix interactions in fibrosis

Cell–matrix interactions can be modelled in vitro using single cell types on reductive 2D elastic substrates with tunable stiffness or adhesive ligand presentation. Although such 2D models have revealed various

effects of the ECM on stromal, immune and stem cell behaviour in fibrosis, they do not faithfully capture the mechanical and topographical 3D environments that cells must negotiate[10,18]. For example, native ECM is not homogeneous, but rather composed of heterogeneous aggregates of ECM proteins (such as collagen, elastin, hydrated gels of glycosaminoglycans and proteoglycans) organized into fibrils, sheets and porous networks. In fibrosis, homeostatic ECM is transformed over time through deposition, crosslinking and proteolysis of matrix proteins, often resulting in dense, aligned collagen fibrils (for example collagen type I, III and V in skin scarring). Furthermore, univariate properties, such as substrate stiffness, do not accurately represent the time-dependent and nonlinear viscoelastic behaviour of native and fibrotic ECM, such as slip, creep and stress relaxation under deformation. These dissipative material properties have important and independent effects from bulk stiffness on fibroblast and stem cell differentiation, migration, matrix remodelling and mechanical activation by altering engagement of focal adhesions and force-gated ion channels[87,88]. Illustrating the importance of such native matrix cues on fibrotic cellular behaviour, primary lung fibroblasts cultured in decellularized matrices derived from patients with idiopathic pulmonary fibrosis recapitulate gene expression signatures of the disease and enter positive feedforward loops of fibrotic activation[89].

Alternatively, biomaterials with tunable mechanical and topographical properties (such as stiffness, stress relaxation rate and porosity) derived from chemically modified natural biopolymers, such as gelatin, dextran, alginate and hyaluronic acid, or synthetic polymers, such as poly(ethylene glycol) (PEG), poly(lactic-*co*-glycolic acid) (PLGA) and poly(ε-caprolactone) (PCL), can be applied to model fibrotic ECM. For example, collagen matrices with tunable fibre thickness, packing and pore size, which can mimic the microarchitecture of fibrillar collagen networks, were applied to show that changes in local strain-stiffening properties with increasingly bundled fibres and wider void spaces may modulate myofibroblast cell–matrix adhesion efficiency and actomyosin contractility independently of bulk stiffness[90,91]. Photolithographical patterning and bioprinting of hydrogel substrates with physiologically relevant stiffness gradients have been applied to study durotaxis, that is, the migration of cardiac, hepatic and other myofibroblasts towards stiffer matrix regions, probably through preferential maturation of focal adhesions and Rac-mediated cytoskeletal reorganization at the leading edge[92]. Although durotaxis remains challenging to investigate in vivo in the context of fibrosis, it may be relevant immediately following injury, as increased vascular permeability in response to inflammatory cytokines promotes third-space fluid accumulation, thereby stiffening the interstitial space and increasing recruitment of surrounding epithelial, endothelial and immune cells[38].

Light-activated stiffening hydrogel systems can mimic the dynamic 'waxing and waning' mechanical properties of fibrosis in situ[93], revealing stronger activation (α-SMA expression, YAP nuclear translocation) of cardiac[75] and hepatic stellate[94] myofibroblasts following preculture in softer conditions; thus, softer matrix conditions at homeostasis may serve to prime the fibrotic stromal response. Furthermore, fibroblast migration patterns have been analysed on bioengineered substrates, revealing long-range transmission of mechanical forces through connective tissue networks. In a process termed 'paratensile signalling', myofibroblasts can activate fibroblasts hundreds of micrometres away nearly instantaneously by transmitting contractile forces down aligned collagen fibres[95]. In addition, viscoelastic properties of the ECM, such as strain stiffening and stress relaxation, evolve over the course of disease, augmenting such long-range force transmission to promote expansion of the fibrotic 'front'[96].

Therefore, biomaterials that can mimic the properties of native fibrotic matrix (for example heterogeneity, anisotropy and viscoelasticity) enable the study of reciprocal cell–matrix interactions that incite and propagate fibrotic processes to identify mechanotransduction signalling mechanisms that may be therapeutically leveraged (Fig. 2).

Modelling cell–cell forces in fibrosis

Cell and molecular engineering technologies allow the manipulation and quantitative analysis of cell–cell mechanical forces in models of fibrotic disease. In particular, FRET-based biosensors can be applied to measure cell–cell mechanical forces in live cells, owing to their sensitivity to intermolecular distance and orientation[97]. For example, genetically engineered catenins that contain intra-protein FRET donor and acceptor moieties can report on mechanically induced conformational changes within adherens junctions[97,98]. Similar conformational-change-based FRET moieties have been designed for vinculin, E-cadherin and other proteins present at cell–cell interfaces[97,99]. For example, FRET-modified Rho GTPases revealed less GTPase activity at cell–cell interfaces in stiff polyacrylamide–collagen hydrogels, mimicking fibrotic tissue, compared with soft hydrogels[100]. Similarly, using FRET-conjugated desmoglein-2 (DSG-2), a component of cardiomyocyte–cardiomyocyte desmosomes that has been posited to inhibit fibrogenic processes in the heart, DSG-2 was shown to directly mediate the transmission of cell–cell tensile forces in vitro[101,102].

Cell–cell forces in fibrosis can also be measured by single-cell atomic force microscopy, for example to quantify fibroblast–fibroblast and endothelial cell–fibroblast adhesion forces during endothelial-to-mesenchymal transition[103]. Although not yet widely applied to the study of fibrosis, synthetic Notch (synNotch) receptors and optogenetically modified cadherin–catenin complexes may also be used to probe forces at the cell–cell interface[104–106]. SynNotch receptors, for example, can be programmed to produce quantifiable fluorescence in response to predefined cell–cell contacts, and optogenetics may enable spatiotemporally defined photoactivation of cell–cell adhesion-related signalling pathways[104–106].

Moreover, 3D printing may enable the precise spatial patterning of cells and/or biochemical ligands for the study of cell–cell mechanical interactions, modelling of drug-induced fibrosis, and screening of putative antifibrotic therapies. For example, cirrhosis in response to trovafloxacin-induced injury can be modelled in printed liver models that contain spatially patterned hepatocytes, HSCs and endothelial cells[107]. Similarly, cardiomyocytes and cardiac fibroblasts can be 3D-printed with variable spatial concentration to mimic the onset of localized fibrotic regions following myocardial infarction and to test pro-regenerative microRNAs[108]. Thus, accurate quantification of cell–cell mechanical forces may benefit from combinations of in situ biosensors, such as FRET systems, with high-resolution spatial fabrication techniques, such as 3D printing.

Modelling fluidic and osmotic forces in fibrosis

Two-dimensional fluidic flow models have yielded fundamental insights into the cellular machinery by which endothelial, hepatocyte, immune and other cell types respond to homeostatic and disturbed fluidic flow conditions[109]. However, 2D flow model systems do not fully capture the dynamic 3D fluidic microenvironments in fibrotic disease states that often involve interactions between multiple dysfunctional cell types. For example, a hallmark of liver fibrosis

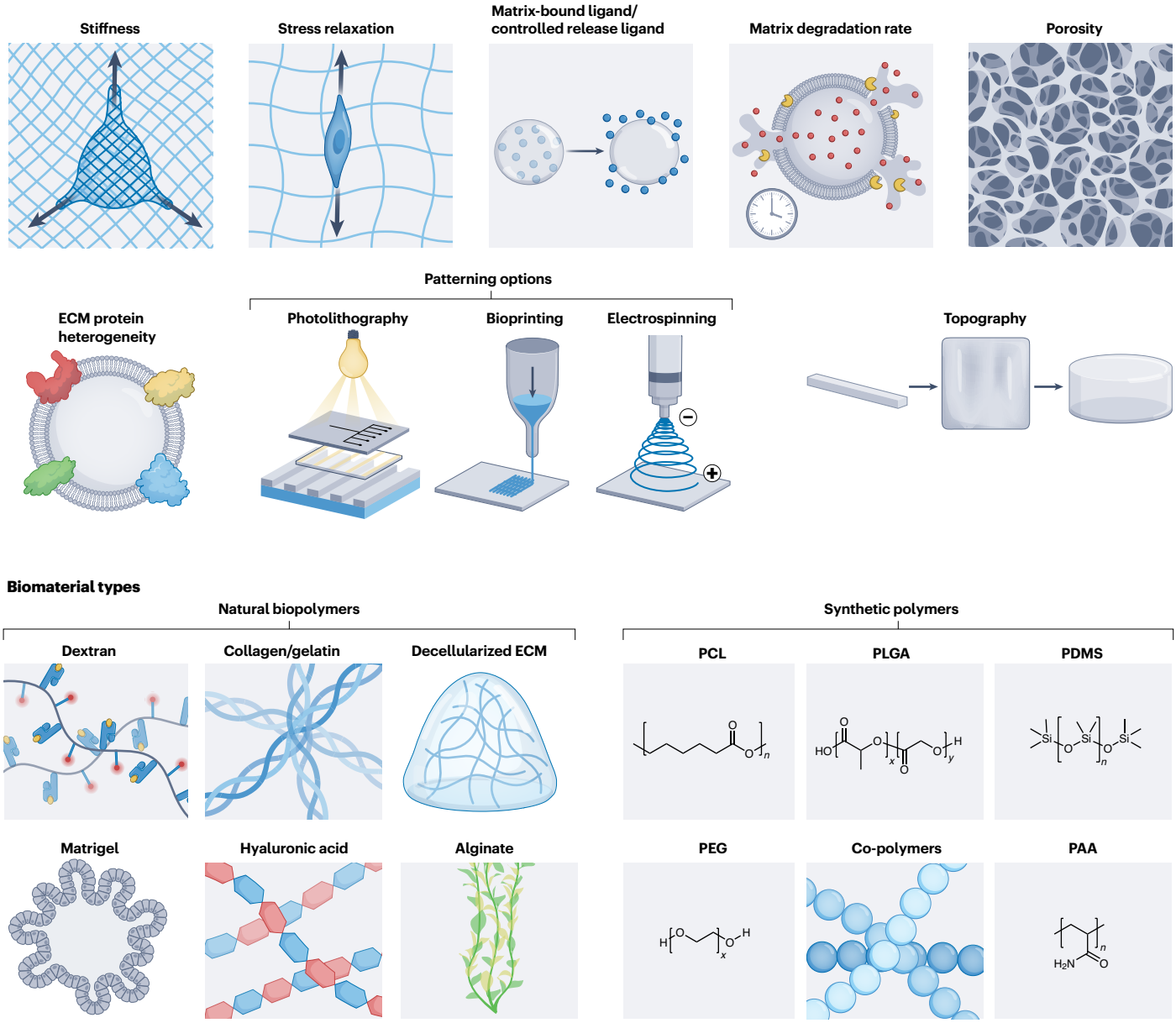

Fig. 2 | Biomaterials for the treatment and modelling of fibrosis. The biophysical parameters of biomaterials can be modified for the design of models and treatments of fibrosis, including magnitude and directionality of stiffness, stress relaxation of biologically relevant substrates, incorporation of covalently matrix-bound or physically encapsulated soluble ligands, topography and porosity of native tissue matrix, and spatial patterning of biochemical and biophysical factors. Common biomaterials used in the context of fibrosis include natural biopolymers, such as polysaccharides (for example dextran, hyaluronic acid), proteins (for example collagen, gelatin) and multicomponent mixtures (for example decellularized matrix, Matrigel). Synthetic polymers, such as poly(lactic-*co*-glycolic acid) (PLGA), poly(ε-caprolactone) (PCL), poly(ethylene glycol) (PEG) and various co-polymers can be applied for therapeutic applications and in vitro modelling of fibrosis. ECM, extracellular matrix; PAA, poly(acrylic acid).

is dysfunction of the sinusoidal endothelium, which stimulates HSC conversion to myofibroblasts and accumulation of matrix proteins in the perisinusoidal space, thereby increasing intrahepatic resistance to flow and shear stress, altering oxygen and nutrient flow to hepatocytes, and inducing further endothelial dysfunction via Notch activation[49]. Sinusoid-on-a-chip platforms that contain layered human sinusoidal endothelium, hepatocytes, Kuppfer immune cells and a collagen gel can collectively simulate the sinusoidal basement membrane and its surrounding microenvironment, and physiologic levels of shear flow can be replicated within polydimethylsiloxane-based microfluidic channels[110,111]. Under low shear stress conditions, these platforms can maintain primary hepatocyte, endothelial cell and HSC phenotypes

up to 4–5 weeks, including albumin, cytochrome P450 family protein and hepatocyte growth factor expression[48]. However, an increase in shear stress (>2 dyn cm^{-2}) or culture of endothelial components on stiff matrix substrates (36 kPa, mimicking the upper limit of measured cirrhotic liver elastic moduli) causes hepatocyte dysfunction and endothelial 'capillarization' with obliteration of fenestrae, mimicking the initial steps of liver fibrosis[112,113].

Two-dimensional microfluidic model systems, that is, organomimetic devices that enable control of dynamic fluid flow over organelles, cells and tissues, have been applied to study the response of renal tubular epithelial cells, podocytes and endothelial cells to high shear rates (>0.25 dyn cm^{-2}); however, the relevance of these studies to fibrotic progression and disruption of the glomerular basement membrane in conditions such as hypertension remains unclear, as these systems produce isolated 2D shear forces that may not fully recapitulate the mechanical microenvironment that these cells experience in vivo[114–116]. Kidney-on-a-chip platforms containing cocultured renal tubular epithelium and peritubular capillary endothelium within multichamber microfluidic devices allow the investigation of cellular responses to fluidic forces and osmotic gradients in the kidney, demonstrating that the endothelial barrier loses its selectivity toward proteins and drugs at high shear rates, in addition to changes in actin cytoskeletal organization, PECAM expression and von Willebrand factor expression[117]. Similar organ-on-a-chip platforms can simulate fibrosis in cardiac and lung tissues, enabling modelling of hemodynamic processes beyond shear stress, such as compressive forces, cyclic stretch and pressure overload[118–120]. Importantly, such platforms allow non-invasive analytical assessment, for example transepithelial electrical resistance measurements to infer cell barrier integrity, and light sheet fluorescence microscopy[121]. However, microfluidic systems to study fluidic forces in fibrotic diseases at the organelle scale (for example cilia, microvilli) remain to be developed. This may be achieved through organoid technology; for example, motile ciliated epithelium can be generated using suspension-based culture systems to eliminate ECM-based biochemical and biophysical cues that restrict epithelial polarity, thereby forming engineered 'inside-out' alveolar organoids that mimic mucociliary clearance functions[122].

Responses to fluidic forces are greatly influenced by dimensionality (that is, 2D versus 3D flow environments), cell–matrix interactions and cell–cell interactions, through signalling machinery (that is, integrins, ion channels) and interdependent effects (that is, polarity-enforcing interactions with the basement membrane influence cell–cell junction protein expression). Thus, care should be taken in inferring independent effects of flow-mediated mechanotransduction signalling mechanisms in fibrosis.

Modelling physical confinement and nuclear mechanotransduction

Engineered models of physical confinement in fibrosis have primarily focused on modulating fibroblast shape, aspect ratio and size through changes in scaffold porosity and architecture, as well as variations in the density and geometric distribution of cell-adhesive ligands[74,76,123]. For example, fibronectin-coated micropatterns can be applied to modify the nuclear volume in 3T3 fibroblasts by altering the density and geometric distribution of fibronectin ligands. Here, physical compaction decreases nuclear volume and reduces histone acetylation, which can be recapitulated using profibrotic HDACs[123]. Physical confinement of fibroblasts and other cell types can also be modelled by the direct application of compressive forces to cultured cells[74,124,125], for example by seeding cells within flexible polydimethylsiloxane constructs modified with cell-adhesive ligands such as fibronectin. The cells can then be analysed using nuclear dyes, such as Hoechst 33342, and/or live-cell imaging to observe the impact of applied forces on nuclear shape and size[74,124,125].

The LINC complex plays a central role in mediating profibrotic epigenetic modifications that occur downstream of physical perturbation[74,76,123]. To investigate the effects of nuclear perturbations on epigenome changes, nuclear stiffness can be quantified in response to applied tension[74,126] using isolated nuclei with intact transmembrane LINC cultured in scaffolds with magnetic beads or other mechanically responsive biomaterials.

Targeting mechanical forces in fibrosis

Targeting cell–matrix forces

Molecular therapies. As our understanding of the molecular and cellular mechanisms underlying fibrosis of various organs has expanded, so too has the repertoire of therapeutics to mitigate fibrosis and, in some cases, reverse fibrotic disease. Most therapeutics approved or under clinical investigation for mitigating fibrosis are small molecules or antibodies targeted against myofibroblasts, ECM components, crosslinking enzymes, inflammatory cytokines or cells. For example, pirfenidone is a small molecule with activity against TGFβ signalling that broadly inhibits the myofibroblast phenotype, which was approved along with nintedanib (a broad inhibitor of angiogenic growth factors) to treat idiopathic pulmonary fibrosis, albeit with variable efficacy[127]. Targeted inhibition of dipeptidyl peptidase 4 (DPP4), highly expressed by Engrailed-1 lineage-positive fibroblasts, also mitigates fibrosis in mouse models of wound healing and scleroderma, possibly by preventing degradation of the proangiogenic cytokine stromal cell-derived factor 1 (SDF-1)[128,129]. Agents targeting the ECM include GS-6624 and STX-100, which are monoclonal antibodies against the matrix crosslinking enzyme lysyl oxidase-like-2 and integrin αvβ6, respectively[130–132]. Monoclonal antibodies against TNF (for example etanercept), IL-1 (anakinra), IL-4/IL-13 (dupilumab) and other inflammatory cytokines have also been studied in clinical trials for idiopathic pulmonary fibrosis, systemic sclerosis and keloids[132]. Various clinical trials for molecular therapies targeting myofibroblasts, ECM components and inflammatory signalling mechanisms have been completed, but few have successfully reached their primary endpoints (Table 1).

Mechanotransduction signalling mechanisms may represent a new frontier for the clinical translation of therapeutics to prevent or reverse fibrotic disease. In particular, mechanotransduction inhibitors targeting the FAK–ROCK–YAP signalling axis may serve as antifibrotic therapies. For example, inhibition of FAK and YAP signalling with VS-6062 induces regeneration following injury in mouse and pig models of wound healing, with recovery of normal skin adnexal structures (hair follicles, glands) and matrix ultrastructure[133]. The YAP inhibitor verteporfin significantly reduces fibrosis in an ischaemia-dependent cardiac mouse fibrosis model[134], and FAK inhibition in both cardiomyocytes and myofibroblasts reduces fibrosis post-myocardial infarction in animal models through downregulation of mTOR and ERK1/2 signalling[135,136]. Finally, indirect modulation of YAP signalling through dopamine receptor inhibition reverses chemically induced liver fibrosis in a mouse model[137]. Although in the preclinical phase, these mechanotransduction signalling-targeted therapies may not only reduce fibrosis in response to organ injury, but also promote regenerative healing responses and, in some cases, reverse pre-existing fibrosis.

Table 1 | Molecular and biomaterial-based approaches for the treatment of fibrosis

Therapy	Target or material	Compound	Molecular target	Indications	Status
Molecular therapies	Myofibroblasts	Pirfenidone	TGFβ	Lung, liver, renal, cardiac fibrosis	Clinic
		Nintedanib, imatinib	FGFR/VEGFR/PDGFR	Nephrogenic systemic fibrosis, lung fibrosis	Clinic
	Extracellular matrix	Sitagliptin	DPP4	Systemic sclerosis, scarring, liver fibrosis, renal fibrosis	Preclinical[204–206] NCT01963845[a] (ref. 207)
		VS-6062	FAK	Scarring, liver, lung fibrosis	Preclinical[133,208–210]
		Fasudil, Y-27632	Rho/ROCK	Lung fibrosis	Preclinical[211,212]
		Verteporfin, statins, dihydrexidine	YAP/TAZ	Scarring, liver fibrosis, lung fibrosis, radiation fibrosis	Verteporfin: preclinical[24,25] Statin: NCT05832229, NCT04971577, NCT00255242[a] (refs. 213–215), NCT04385433, NCT01268202[b] (refs. 216,217) Dihydrexidine: preclinical[137]
		PF-06473871, EXC 001, FG-3019	CTGF	Scarring, lung fibrosis, liver fibrosis	PF-06473871: NCT01730339 (ref. 218) EXC 001: NCT01037985[a] (ref. 219) FG-3019: NCT01890265[b], NCT04419558, NCT01217632[a] (refs. 220–222)
		GS-6624	Lysyl oxidase-like-2	Lung fibrosis, liver fibrosis	NCT01759511[a], NCT01769196[a] (refs. 223,224) NCT01672879[a], NCT01672866[a], NCT01707472[a] (refs. 225–227)
	Inflammation	IDL-2965, PLN-74809, GSK3008348, STX-100	Integrin αvβ1/β3/β6	Lung fibrosis, renal fibrosis	IDL-2965: NCT03949530[a] (ref. 228) PLN-74809: NCT04396756 (ref. 229) GSK3008348: NCT02612051[b] (ref. 230) STX-100: NCT01371305[a], NCT00878761[a] (refs. 231,232)
		Etanercept	TNF	Lung fibrosis	NCT00063869[a] (ref. 233)
		Anakinra	IL-1	Lung, joint fibrosis	Clinic
		Tocilizumab	IL-6	Lung fibrosis, systemic sclerosis	NCT02453256[b] (ref. 234)
		Tralokinumab, Dupilumab, lebrikizumab, QAX576	IL-4/IL-13	Keloids, lung fibrosis	Dupilumab: NCT04988022, NCT05128383 (refs. 235,236) QAX576: NCT00987545[a] (ref. 237) Tralokinumab: NCT01629667[a] (ref. 238) Lebrikizumab: NCT01872689[a] (ref. 239)
		Carlumab	CCL2	Lung fibrosis	NCT00786201[a] (ref. 240)
		PRM-151	Monocyte differentiation (Pentraxin-2)	Lung fibrosis, myelofibrosis	NCT02550873[b] (ref. 241) NCT01981850 (ref. 242)
		Sivelestat	Neutrophil elastase	Lung fibrosis, ARDS	Clinic (unclear benefit in phase 4 trials)
Biomaterials	Decellularized ECM	—	—	Scarring, cardiac fibrosis, liver fibrosis	Clinic (e.g. Alloderm) NCT02305602 (ref. 243) Preclinical[244]
	Polymeric hydrogels	Gelatin/collagen-based	—	Scarring, diabetic/chronic wounds, adhesions, multiple fibroses	Preclinical[141,142,245]
		Dextran-based	—	Scarring, cardiac fibrosis	Preclinical[139] Preclinical[246]
		Hyaluronic acid-based	—	Scarring, adhesions	NCT00958425[a] (ref. 247) NCT02166554[b], NCT05360186 (refs. 248,249)
		Alginate-based	—	Cardiac fibrosis	NCT01226563[a], NCT01311791[b] (refs. 250,251)
		3D-printed	—	Scarring, diabetic/chronic wounds, adhesions, multiple fibroses	Preclinical[138]

Trial identification numbers are provided for targets that are currently undergoing or have previously undergone clinical trials. [a]Trials failed to meet their primary endpoint, were discontinued early for futility or technical limitations, or do not have results posted or published. [b]Studies successfully met their primary endpoint. ARDS, acute respiratory distress syndrome; CCL, chemokine ligand; CTGF, connective tissue growth factor; DPP4, dipeptidyl peptidase 4; FAK, focal adhesion kinase; FGFR, fibroblast growth factor receptor; IL-1, interleukin-1; IL-4, interleukin-4; IL-6, interleukin-6; IL-13, interleukin-13; PDGFR, platelet-derived growth factor receptor; ROCK, Rho-associated protein kinase; TGFβ, transforming growth factor-β; TNF, tumour necrosis factor; VEGFR; vascular endothelial growth factor receptor; YAP/TAZ, Yes-associated protein/transcriptional coactivator with PDZ-binding motif.

Engineered biomaterials. Various biomaterials have been developed for the treatment and prevention of fibrosis (Table 1), often designed to recapitulate tissue-regenerative motifs and/or directly inhibit fibrotic progression. For example, pro-regenerative scaffolds can be fabricated from natural biopolymers, such as gelatin, dextran and hyaluronic acid, to provide porous structural support for cell infiltration and tissue regeneration following injury[138–140]. In particular, gelatin and collagen are applied for the treatment of fibrosis in multiple tissues[138]. For example, injection of a collagen matrix shortly after myocardial infarction can inhibit long-term fibrosis by suppressing acute inflammation in murine models[141]. Similarly, gelatin-methacrylate scaffolds promote healthy dermal regeneration in vitro and limit fibrosis owing to their tailored porous architecture[142]. In addition, dextran hydrogels have been developed to treat burn-related scarring[139], and spatially patterned hyaluronic acid hydrogels can promote tissue regeneration in skin scarring and stroke in mouse models[140].

Decellularized ECM contains tissue-specific biochemical cues, which allow de novo tissue development[138,143]. For example, decellularized skin ECM can be implanted to reduce scar contraction in a murine model of burn injury by providing porous scaffolding for dermal regeneration and by presenting skin-derived angiogenic ligands[143]. Similarly, tissue-engineered constructs can be implanted at the site of injury for in situ tissue regeneration or alternatively cultured in vitro to generate tissue for implantation. For example, a construct composed of decellularized liver ECM, autologous cells and SDF-1 can be applied to treat liver fibrosis and promote in situ hepatic regeneration in a rat model[144]. In addition to providing tissue-specific cells and bioactive factors, biomaterials can be applied to promote functional, rather than fibrotic, tissue regeneration by mimicking the healthy tissue's physical architecture and mechanical properties. To this end, 3D printing can produce user-defined spatial organization of antifibrotic cell and material formulations[138]. For example, decellularized skin ECM can be printed to recreate the topological and mechanical properties of healthy dermal matrix and minimize fibrosis upon implantation in murine models[143]. Tissue-engineered constructs can also be engineered with anti-inflammatory compounds to prevent fibrotic progression. For example, 3D-printed decellularized splenic ECM can be combined with delivery of soluble anti-inflammatory IL-10 and other cardioprotective factors for myocardial infarction treatment in mice[145].

In addition to natural biopolymers, various synthetic polymers have been used for the development of antifibrotic scaffolds. Although these materials are typically bioinert, the biochemical and physical properties of synthetic polymers, such as poly(lactic-co-glycolic acid) (PLGA) and poly(ε-caprolactone) (PCL), can be tuned to design them as carriers for antifibrotic drug delivery or scaffolds for cell seeding and infiltration[146,147]. For example, PLGA microspheres can deliver soluble kynurenic acid (which broadly inhibits fibroblast ECM production) for the reduction of dermal fibrosis after acute wounding in a rat model[148]. Nanoparticle-based therapies enable the localized, controlled release of small-molecule inhibitors of the FAK–ROCK–YAP signalling axis, including ROCK inhibition by Y-27632 and YAP inhibition by verteporfin[149,150]. Hybrid PCL–collagen scaffolds can support corneal repair and prevent stromal fibrosis following injury[151], and an electrospun PCL scaffold containing a laminin β1 chain fragment abrogates *MMP2* expression, blocks EMT and rescues peritoneal fibrosis ex vivo and in mouse models[152]. D-chiral peptide-crosslinked microporous annealed particle (MAP) hydrogels can promote skin regeneration with minimal fibrosis by preferentially recruiting IL-33-expressing type 2 myeloid cells[153].

Synthetic biomaterials have the key advantage that biochemical ligand presentation and topographical substrate cues can be modified to tune adhesion, porosity and alignment independently of stiffness. For example, MAP hydrogels contain tunable void spaces to maximize cell infiltration and spreading, thereby accelerating revascularization, reducing inflammation, and promoting migration of progenitor cell populations in injury sites[154,155]. In addition to their controlled release applications, nanoparticle additives of PCL, gold, carbon and other materials can be incorporated into wound healing scaffolds to provide antifibrotic topographical features, possibly by inhibiting integrin activation and subsequent YAP signalling as well as myofibroblast activation[156–158].

Targeting cell–cell forces in fibrosis

Therapeutics that influence cell–cell forces are often designed to target individual proteins within adherens junctions. The expression of cadherins, for example, may be modulated by small-molecule therapeutics to promote the stability of adherens junctions, because their mechanical disruption can release profibrotic factors, such as β-catenin[159]. For example, delivery of the small molecule curcumin increases E-cadherin expression and cell–cell contacts, thereby reducing the severity of renal fibrosis in a rat model[160]. Cadherin-11 also represents a common target for therapeutic inhibition, given its role in transmitting cellular tension within myofibroblast–fibroblast contacts[159]. Accordingly, various cadherin-11 blocking monoclonal antibodies have been explored in animal models for the treatment of dermal fibrosis, pulmonary fibrosis, systemic sclerosis, cardiac fibrosis and more[39,159,161]. Furthermore, a humanized anticadherin-11 antibody, RG6125, has been tested in clinical trials for rheumatoid arthritis, but produced inferior responses compared with the current standard-of-care[162]. Most of these anticadherin-11 antibodies, as well as other small-molecule inhibitors, such as celecoxib, have been designed to competitively inhibit homophilic bond formation through the EC1 domains of cadherin-11, because this homodimerization process mediates fibroblast–fibroblast adhesion[159,163].

β-catenin represents another common molecular target in adherens junctions, given its central role in initiating profibrotic transcriptional programmes, such as EMT, by acting as a transcription factor and/or binding TGFβ and Wnt[159]. The delivery of small-molecule β-catenin antagonists, such as istradefylline, can reverse EMT and inhibit both dermal and pulmonary fibrosis in murine models[164–166].

Most therapeutics that modulate cell–cell forces have been designed to target individual mechanomodulatory proteins within adherens junctions such as cadherin-11 and β-catenin, rather than directly manipulating the cells themselves. Many of these small-molecule and antibody-based therapies have shown positive results in animal models of fibrosis, and a select few have thus far moved forward to clinical trials, albeit with limited efficacy compared with more established antifibrotic treatments, such as TGFβ and TNF modulators[159,163].

Targeting fluidic and osmotic forces in fibrosis

Therapeutics targeting the renin–angiotensin–aldosterone (RAAS) signalling axis (for example ACE inhibitors) directly target fluidic pressure overload and are clinically approved modulators of hypertensive renal and myocardial fibrosis[167]. In addition, antifibrotic therapies are being preclinically explored that target fluidic force mechanotransduction signalling mechanisms. For example, several fluidic force-sensing mechanisms ultimately result in Ca^{2+} influx; accordingly,

Ca^{2+} channel inhibition with nifedipine ameliorates renal fibrosis in chemical injury and transgenic rat models of renal perivascular fibrosis[168,169], but may also exacerbate renal lipogenesis[170]. Alternatively, fluidic force-responsive ion channels may be targeted; for example, inhibition of the cation channel short transient receptor potential channel 3 (TRPC3) with pyrazol-3 mitigates cardiac fibroblast α-SMA expression, ERK phosphorylation and NFAT activation, suppressing atrial fibrillation in large-animal models with tachypacing-induced heart failure[171].

Therapeutics targeting flow and osmotic mechanotransduction mechanisms driving EMT in the kidney, lung and other organs also hold promise as antifibrotic agents. For example, lumacaftor and ivacaftor, which target CFTR chaperoning to the cell membrane to correct transmembrane osmotic gradients, have become standard-of-care in cystic fibrosis, substantially increasing the length and quality of life[172]. Other ionic channels, such as volume-regulated anion channel (VRAC) and chloride intracellular channel 4 (CLIC4), as well as paracellular proteins involved in ion transport, such as claudins, have also been implicated in EMT and targeted to prevent renal fibrosis in rodent models[173]. Finally, pharmacologic inhibition of polycystin-2 (involved in ciliary flow sensing) with triptolide reduces expression of EMT-related markers and deposition of ECM proteins in small-animal models of renal fibrosis[174].

Targeting physical confinement and nuclear mechanotransduction

Physical confinement and cytoskeletal–nuclear mechanotransduction primarily result in epigenetic modifications within the context of fibrotic disease. Therefore, therapeutics have thus far mainly been designed to target individual molecular mediators of the epigenome, in particular, HDACs, which are involved in the fibrosis of multiple organs by accumulating in the nucleus following LINC-mediated nuclear mechanotransduction[78] (Fig. 3). Various HDAC inhibitors have been explored to treat mouse models of cardiac fibrosis following myocardial infarction, including small molecules, such as trichostatin A and Rhein, as well as various siRNAs targeting individual HDACs[175–177]. Similar HDAC inhibitors have been applied for the treatment of non-cardiac fibroses, such as liver cirrhosis; for example, *N*-hydroxy-7-(2-naphthylthio)heptanomide (HNHA) is a small-molecule inhibitor of HDACs, reducing profibrotic activation of both mouse and human HSCs in vitro and suppressing liver fibrosis in a rat model[178]. The selective inhibitor of class IIa HDAC, MC1568, reduces downstream expression of profibrotic genes, such as α-SMA, fibronectin and collagen-1a1 in a mouse model of renal fibrosis[179].

Other small-molecule HDAC inhibitors have demonstrated efficacy in rodent models of fibrotic diseases, including idiopathic pulmonary fibrosis, but few therapeutics have reached clinical trials[180,181]. A major limiting factor in these therapeutic strategies is that HDACs themselves acetylate a variety of non-histone proteins, and the broad inhibition of HDACs can therefore produce widespread and often fatal off-target effects[180]. Furthermore, histone deacetylation plays a crucial role in tissue homeostasis and has oncosuppressive functions, necessitating greater specificity in the targeting of profibrotic HDACs[78].

Beyond HDAC inhibition, the LINC complex can be targeted in the context of nuclear mechanotransduction. For example, adenovirus-based transduction of a genetically modified SUN1 protein disrupts LINC complex formation for the treatment of murine cardiac fibrosis[182]. Similarly, ablation of SUN2 can aid in the treatment of murine cardiac fibrosis; however, this treatment also increases profibrotic HSC activation in models of hepatic fibrosis[80,183]. Thus, similar to the broad inhibition of HDACs, targeting the LINC complex, which mediates a range of homeostatic functions, may produce off-target effects that complicate clinical translation[74].

Outlook

Multiomics studies of fibrosis and wound healing

Single-cell RNA sequencing (scRNA-seq) has revealed new insights into the contributions of mechanotransduction signalling and mechano-activated cell subpopulations to fibrotic disease progression[184]. Nevertheless, scRNA-seq does not inherently capture spatiotemporal phenomena, which include the contributions of spatially defined cellular communities and/or transient biological processes. To this end, spatial transcriptomics and multiplexed immunohistochemistry can resolve the spatial heterogeneity of fibrosis by quantifying in situ gene and protein expression, which may help to identify cell–cell interactions involved in mechanotransduction signalling and elucidate how localized mechanobiological niches influence profibrotic transcription[185,186]. Sampling of multiple timepoints from animal model and/or human disease progression may furthermore reveal how mechanobiological factors evolve during temporally distinct stages of fibrosis, such as initial injury, inflammation, fibrotic ECM deposition and post-fibrotic remodelling[25,187,188]. However, transcriptomic characterization alone is insufficient to characterize the mechanisms of fibrotic progression. Epigenomic techniques, such as single-cell assay for transposase-accessible chromatin using sequencing (scATAC-seq), can be applied to study how epigenetic adaptations, such as changes in chromatin accessibility, are influenced by mechanobiological factors[189,190]. Therefore, multiomics analysis, integrating transcriptomic, epigenomic and other data, may be required to uncover the full biological landscape of fibrotic disease progression[25,189,191] (Box 2). In addition, computationally integrating published omics datasets may reveal cell phenotypes and mechanotransduction pathways involved in the fibrosis of multiple organs[192,193] (Fig. 4).

Next-generation models

The FDA Modernization Act 2.0 and analogous EMA measures have underscored the translational relevance of in vitro models for fibrotic disease modelling and drug testing[85]. In particular, multiorgan-on-a-chip systems and 3D-printed tissue models may be able to capture the spatiotemporal complexity of mechanical forces in fibrosis, for example, by connecting multiple organ models through microfluidic channels that mimic systemic blood flow[194]. These systems enable the modelling of organ–organ interactions, a key factor in many organ fibroses, and may also more accurately capture systemic responses to drug treatments and mechanical perturbation compared with single organ-on-a-chip systems[194,195]. 3D-printed tissue models of fibrosis may recapitulate the precise spatial distributions of cells, ligands and mechanical substrates involved in fibrotic disease; however, this will require improvements in printing resolution and the ability to process and print multiple inks in tandem[196]. Furthermore, 3D-printing strategies may take direction from spatial transcriptomics and other spatial phenotyping technologies, which have uncovered high-resolution spatial blueprints of cell populations and biochemical ligands involved in organ fibrosis.

Importantly, fibrotic microenvironments may be more effectively recapitulated in 3D biomaterial constructs than on 2D substrates. For example, hydrogel crosslinking and stiffness produce inverse effects on pulmonary myofibroblast differentiation in 2D versus 3D substrates; here, 3D models of tunable fibrous topography more accurately

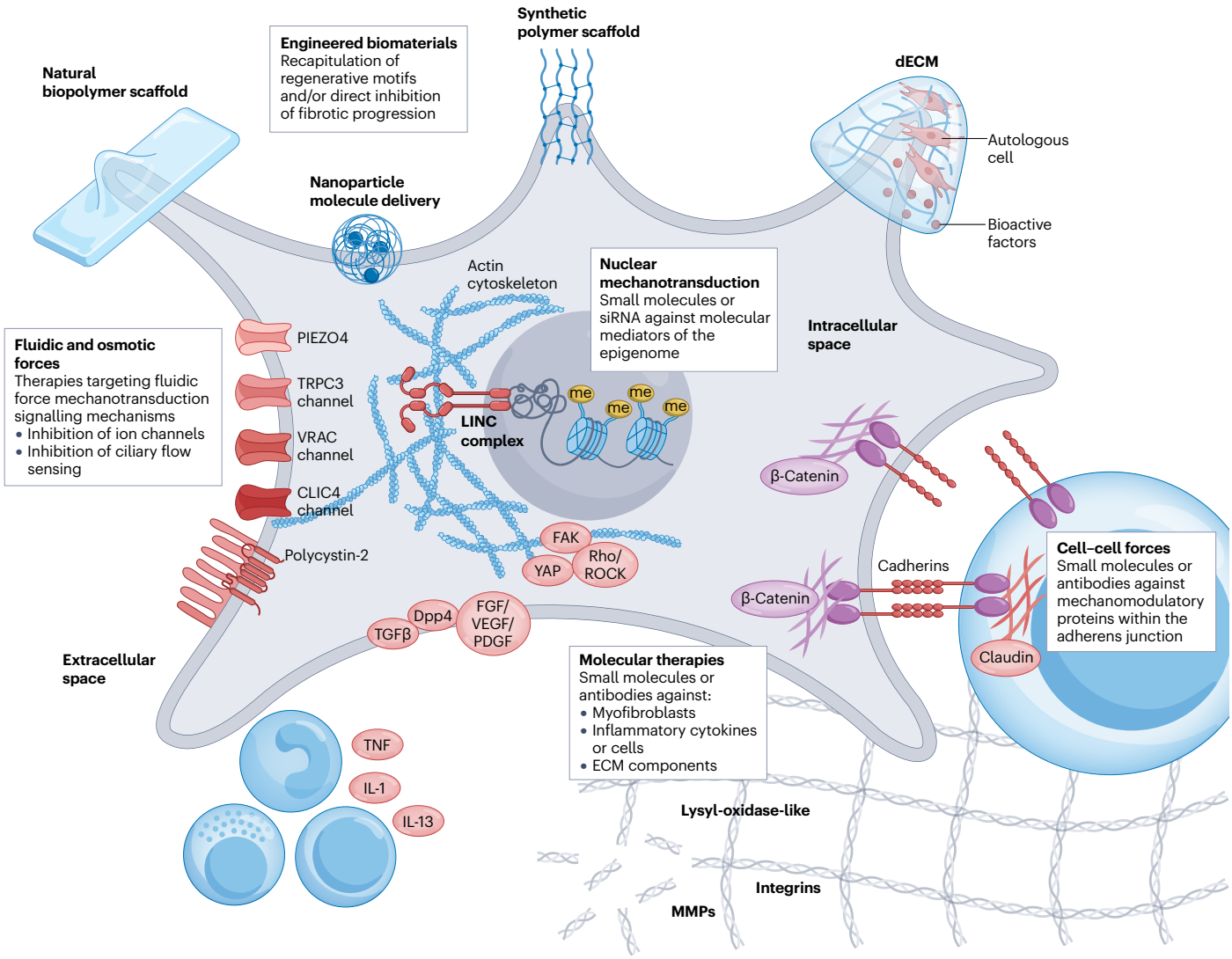

Fig. 3 | Mechanotransduction signalling targets in fibrosis. Various small-molecule and biomaterial-based therapies have been developed to modulate extracellular and intracellular mechanotransduction mechanisms. Most clinically approved or therapeutics in phase 2/3 clinical trials for fibrosis of the skin, lungs and other organs target the myofibroblast phenotype (for example transforming growth factor β (TGFβ) signalling), extracellular-matrix (ECM) crosslinking (for example lysyl-oxidase-like proteins), ECM breakdown (for example matrix metalloproteinases (MMPs)), and adhesion (for example integrins), or inflammation, but have shown variable efficacy. Alternate approaches include targeting central mechanotransduction signalling mediators, such as focal adhesion kinase (FAK) or Yes-associated protein (YAP), fluid and osmotic force-sensing proteins (for example transient receptor potential (TRP) and PIEZO channels), or cell–cell junction proteins (for example cadherins, β-catenin). Biomaterials-based approaches, such as natural and synthetic biopolymer-based hydrogels with variable stiffness and viscoelastic properties, nanoparticle drug delivery systems, and decellularized ECM have shown promise in preclinical and early clinical trials, probably owing to their biomimetic mechanical and topographical properties. dECM, decellularized extracellular matrix; DPP4, dipeptidyl peptidase 4; ESC, embryonic stem cell; FGF, fibroblast growth factor; FRET, Förster resonance energy transfer; HA, hyaluronic acid; iPSC, induced pluripotent stem cell; IL, interleukin; LINC, linker of nucleoskeleton and cytoskeleton; Me, methyl; PDGF, platelet-derived growth factor; Rho/ROCK, Rho-associated protein kinase; VEGF, vascular endothelial growth factor.

reproduce in-vivo- and human-relevant mechanisms of myofibroblast activation, involving MMP-dependent matrix remodelling[197]. Thus, compliant and degradable 3D substrates may actually support fibrogenesis without requiring increased matrix stiffness, challenging prior findings in 2D models[197]. Such 3D biomaterial constructs may help to reveal new fibrogenic mechanisms by more accurately mimicking the mechanical and topographical features of native tissue than 2D models.

Next-generation biomaterials

In addition to advanced fabrication methods, fibrosis models will greatly benefit from the development of biomaterials that recapitulate the precise biochemical and/or mechanical properties of fibrotic ECM. Although synthetic polymers, such as PLGA, PEG and PCL, are commonly applied for the treatment and modelling of fibrosis, these polymers are bioinert and limited in their tunability[146,147]. Additionally,

they are highly adsorbent to proteins, complicating the presentation of specific biochemical cues to cells[198]. These pitfalls may be addressed by new synthetic polymers and biofunctionalized synthetic–natural polymer hybrids that mimic the features of collagen and other fibrotic ECM components. For example, synthetic polyisocyanide hydrogels, forming 3D fibrillar networks, can mimic complex mechanical phenomena of collagen, such as strain stiffening and compression softening[199]. Unlike collagen hydrogels, this synthetic polymer system may also be immune to proteolytic cleavage, which may enable long-term culture for the study of fibrotic disease progression[199]. Bioconjugation strategies may also offer a toolset for the functionalization of synthetic polymers with biochemical cues relevant to the study of fibrosis. For example, hybrid synthetic–natural polymers consisting of PEG conjugated to either healthy or fibrotic decellularized lung ECM through Michael addition allow modular and independent tuning of the resulting polymer's mechanical properties and biochemical composition[200]. Ultimately, next-generation biomaterials may benefit from a variety of bioconjugation tools, including click chemistry and decellularized ECM functionalization, to capture the mechanical and biochemical features of native fibrotic matrix[138,200,201].

Box 2

Measuring fibrosis

Fibrosis is typically 'quantified' using standardized histological preparations of extracellular matrix (ECM), such as Trichrome, Picrosirius red, reticulin silver and collagen immunofluorescence staining. Although amenable to comparison across specimens, subjective analyses of histopathologic images suffer from poor inter- and intra-rater reliability even in the hands of experienced pathologists[255]. Comparison of fibroses either grossly (on a 'visual analogue scale' as in skin scars) or by histopathology (for example cirrhosis) is therefore subject to bias. The clinical translation of antifibrotic therapies has benefited from non-invasive measurement methods, such as FibroScan, in which ultrasound elastography is applied to infer severity of liver fibrosis; however, similar assays are lacking for most other forms of fibrosis[256].

Machine learning may be a promising tool for the quantitative analysis of organ fibroses, whereby images of tissues in homeostasis and varying degrees of fibrotic change can be used to train unsupervised algorithms for automated scoring of disease severity[257,258]. Approaches combining ECM and cellular interaction patterns, clinical metadata and omics data are increasingly being used to establish new histopathological signatures of fibrotic disease[258,259].

In particular, unsupervised models combining transcriptomic, proteomic and matrix ultrastructural data can distinguish biological signatures of wound regeneration and scarring[25,260]. Unsupervised machine learning algorithms can also be trained on image and omics data to identify interaction networks in the foreign body response[261], atrial fibrillation[262] and bone formation in implanted ceramic scaffolds[261]. Given the complexity and heterogeneity of fibrotic tissue microenvironments, the identification of new antifibrotic targets may depend on such multivariate analyses of individual cellular subpopulations and their many interactions.

A range of micromechanical and biochemical sensors have been designed to measure mechanical forces in fibrosis in vitro. At the protein level, optical and magnetic tweezers as well as Förster resonance energy transfer (FRET) sensors can quantify forces in cytoskeletal proteins, ion channels and adherens junctions in the piconewton range[263,264]. At the cell level, atomic force microscopy, micropipettes and micromechanical systems allow the precise quantification of nanonewton-range forces, and traction force microscopy and micropost deformation assays enable indirect quantification of cell–matrix mechanical forces[265]. However, such protein- and cellular-scale forces cannot yet be assessed in situ and in vivo.

Tissue mechanical properties and fibrosis

Regenerative outcomes can be achieved by presenting 'homeostatic ECM' to cells within the injury site (for example healthy decellularized ECM, MAP hydrogels), whereas positive fibrotic feedback loops may be triggered by exposing healthy cells to diseased ECM (for example decellularized lung ECM from patients with idiopathic pulmonary fibrosis). Thus, the question remains whether stiffness, viscoelastic and topographical properties of fibrotic ECM are a consequence or root cause of fibrotic disease progression. Evidence from animal models suggests that changes in the mechanical, biochemical and topographical properties of ECM may precede deposition of scar collagen; by measuring the shear storage modulus of rat livers treated with carbon tetrachloride, for example, it was shown that immediate increases in bulk tissue stiffness precede histologically apparent matrix deposition. Importantly, stiffness and amount of fibrosis did not correlate in this study[202]. Supporting the notion that tissue stiffness may not necessarily be related to bulk collagen deposition, normalizing nanoscale collagen fibril size and crosslinking (but not overall amount of collagen deposited or overall architecture) through lysyl oxidase inhibition in human tissue ex vivo and mouse models of pulmonary fibrosis was sufficient to prevent increases in tissue stiffness[203]. These findings have implications for studies relying on hydroxyproline assays or other experimental readouts quantifying total collagen content as a marker of fibrotic change (Box 2). Moreover, they suggest that fibrotic diseases may be initiated and propagated through a bistable switch in fibroblast phenotype, whereby pathologic stimuli (such as inflammation, osmotic stress and pressure overload) induce early changes in the nanoscale mechanical and topographical properties of the ECM (for example changes in fibre diameter and packing, porosity, slip, strain stiffening and crosslinking) that activate fibrogenic programmes, increase collagen deposition and crosslinking, and stiffen the bulk ECM, thus forming a self-sustaining positive feedback loop. By this paradigm, targeting fibroblast activation without addressing the underlying nanoscale derangements of ECM architecture disrupting 'mechano-homeostasis' is unlikely to achieve long-term resolution of fibrosis, perhaps explaining the limited clinical benefit of approved antifibrotic therapies, such as pirfenidone.

Only few efficacious disease-modifying therapies exist for fibrotic diseases. Mechanical forces are intimately tied to the initiation and progression of fibrosis and thus may represent an important therapeutic target. Bioengineered culture systems, multiomics, genetic engineering, machine learning and other technologies (Box 2) have enabled deeper insight into mechanotransduction signalling and mechanoresponsive cell populations responsible for fibroses. These efforts have, in turn, enabled new molecular (for example FAK, YAP signalling) and biomaterials-based (for example microporous hydrogels) therapies

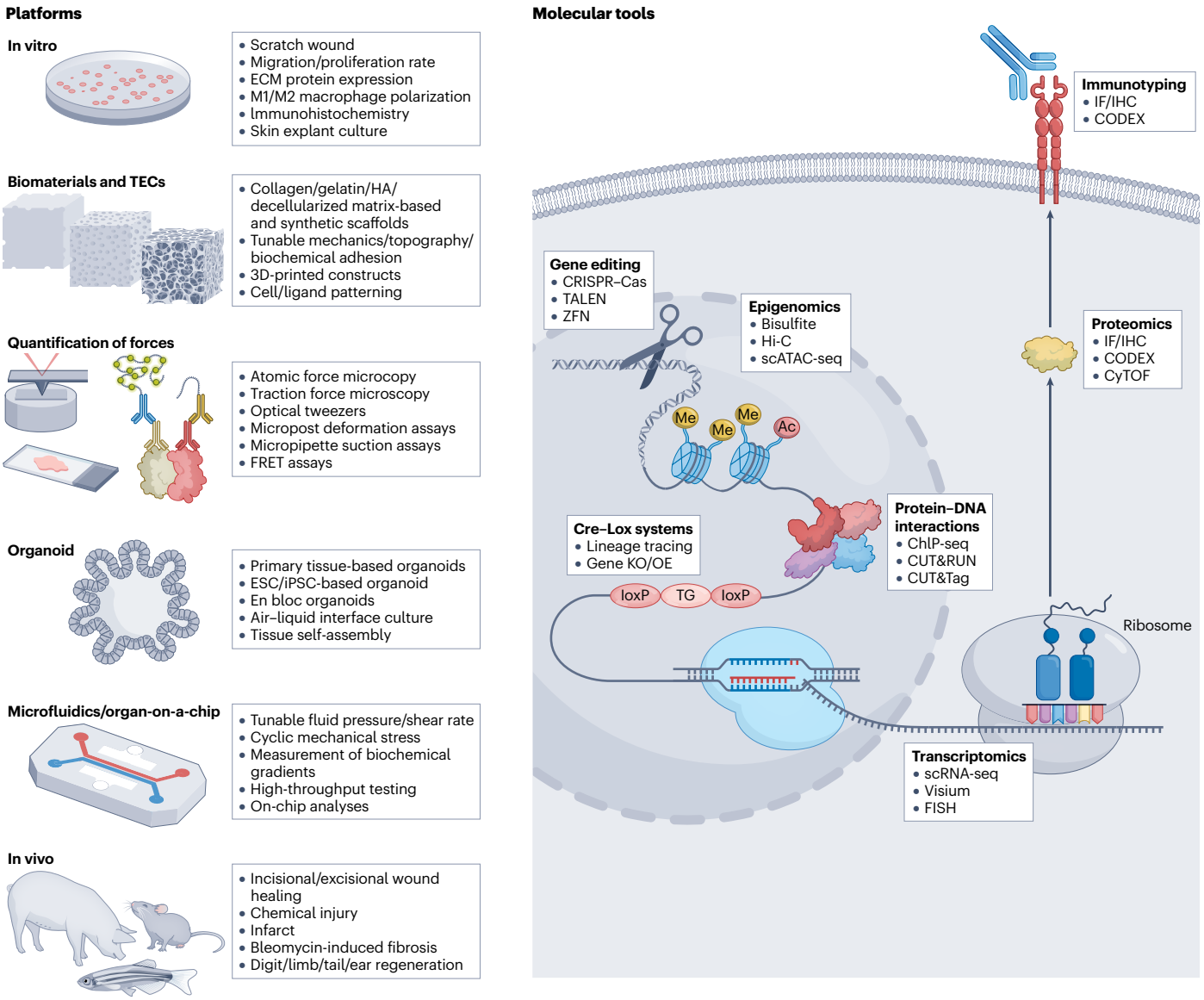

Fig. 4 | Platforms and molecular tools to study fibrosis. Since the advent of the scratch wound assay, substantial progress has been made in modelling fibrotic diseases. Measuring mechanical forces on the pico- and nanonewton scales within fibrotic contexts has been made possible by technologies such as atomic force microscopy, traction force microscopy, optical tweezers, Förster resonance energy transfer (FRET) and micropost deformation assays. In addition, tunable biomaterials, tissue-engineered constructs and organoids can be applied to model the various biochemical, mechanical and microarchitectural features of fibrotic niches. Microfluidic organs-on-a-chip with physiologically distinct compartments can replicate biophysical cues from phenomena, such as fluid shear stress, pressure overload and breathing. In vivo models (for example zebrafish, mice and pigs) of fibrosis can be designed through surgical or chemical injuries. Moreover, molecular tools allow probing of the genomic (for example clustered regularly interspaced short palindromic repeats (CRISPR)), epigenomic (for example assay for transposase-accessible chromatin sequencing (ATAC-seq)), transcriptomic (for example single-cell RNA sequencing (scRNA-seq), Visium) and proteomic (for example cytometry by time-of-flight (CyTOF), co-detection by indexing (CODEX)) landscape of the fibrotic milieu at single-cell resolution. ChIP-seq, chromatin immunoprecipitation followed by sequencing; Cre–Lox, cyclization recombinase–locus of x; ESC, embryonic stem cell; FISH, fluorescence in situ hybridization; HA, hyaluronic acid; Hi-C, high-throughput chromosome conformation capture; IF/IHC, immunofluorescence/immunohistochemistry; iPSC, induced pluripotent stem cell; KO/OE, knockout/overexpression; TALEN, transcription activator-like effector nuclease; ZFN, zinc-finger nuclease.

to prevent and reverse fibrotic disease. The development of therapies will further benefit from identifying the spatiotemporal factors that drive fibrosis (for example spatial multiomics at multiple timepoints), quantitative detection and characterization of fibrotic disease in situ, and next-generation in vitro, ex vivo and in silico models that faithfully recapitulate the complex multicellular dynamics of fibrosis.

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References

1. Bayat, A., McGrouther, D. A. & Ferguson, M. W. Skin scarring. *BMJ* **326**, 88–92 (2003).
2. Atiyeh, B. S. Nonsurgical management of hypertrophic scars: evidence-based therapies, standard practices, and emerging methods. *Aesthetic Plast. Surg.* **31**, 468–492 (2007). Discussion 493–464.
3. Alster, T. S. & Tanzi, E. L. Hypertrophic scars and keloids: etiology and management. *Am. J. Clin. Dermatol.* **4**, 235–243 (2003).
4. Ngaage, M. & Agius, M. The psychology of scars: a mini-review. *Psychiatr. Danub.* **30**, 633–638 (2018).
5. Wynn, T. A. Fibrotic disease and the T(H)1/T(H)2 paradigm. *Nat. Rev. Immunol.* **4**, 583–594 (2004).
6. Gieseck, R. L. 3rd, Wilson, M. S. & Wynn, T. A. Type 2 immunity in tissue repair and fibrosis. *Nat. Rev. Immunol.* **18**, 62–76 (2018).
7. Kaur, A., Mathai, S. K. & Schwartz, D. A. Genetics in idiopathic pulmonary fibrosis pathogenesis, prognosis, and treatment. *Front. Med.* **4**, 154 (2017).
8. Wells, R. G. Tissue mechanics and fibrosis. *Biochim. Biophys. Acta* **1832**, 884–890 (2013).
9. Li, S. et al. Fluid shear stress activation of focal adhesion kinase. Linking to mitogen-activated protein kinases. *J. Biol. Chem.* **272**, 30455–30462 (1997).
10. Wilson, E., Sudhir, K. & Ives, H. E. Mechanical strain of rat vascular smooth muscle cells is sensed by specific extracellular matrix/integrin interactions. *J. Clin. Invest.* **96**, 2364–2372 (1995).
11. Kuipers, A. J., Middelbeek, J. & van Leeuwen, F. N. Mechanoregulation of cytoskeletal dynamics by TRP channels. *Eur. J. Cell Biol.* **91**, 834–846 (2012).
12. Tschumperlin, D. J. et al. Mechanotransduction through growth-factor shedding into the extracellular space. *Nature* **429**, 83–86 (2004).
13. Zeng, Y. et al. CXCR1 and CXCR2 are novel mechano-sensors mediating laminar shear stress-induced endothelial cell migration. *Cytokine* **53**, 42–51 (2011).
14. Hinz, B. Formation and function of the myofibroblast during tissue repair. *J. Invest. Dermatol.* **127**, 526–537 (2007).
15. Alenghat, F. J. & Ingber, D. E. Mechanotransduction: all signals point to cytoskeleton, matrix, and integrins. *Sci. STKE* **2002**, pe6 (2002).
16. Krammer, A., Lu, H., Isralewitz, B., Schulten, K. & Vogel, V. Forced unfolding of the fibronectin type III module reveals a tensile molecular recognition switch. *Proc. Natl Acad. Sci. USA* **96**, 1351–1356 (1999).
17. Martino, F., Perestrelo, A. R., Vinarsky, V., Pagliari, S. & Forte, G. Cellular mechanotransduction: from tension to function. *Front. Physiol.* **9**, 824 (2018).
18. Deng, Z. et al. The extracellular matrix and mechanotransduction in pulmonary fibrosis. *Int. J. Biochem. Cell Biol.* **126**, 105802 (2020).
19. Lu, F. et al. Microdeformation of three-dimensional cultured fibroblasts induces gene expression and morphological changes. *Ann. Plast. Surg.* **66**, 296–300 (2011).
20. Webb, K. et al. Cyclic strain increases fibroblast proliferation, matrix accumulation, and elastic modulus of fibroblast-seeded polyurethane constructs. *J. Biomech.* **39**, 1136–1144 (2006).
21. Dallon, J. C. & Ehrlich, H. P. A review of fibroblast-populated collagen lattices. *Wound Repair. Regen.* **16**, 472–479 (2008).
22. Aarabi, S. et al. Mechanical load initiates hypertrophic scar formation through decreased cellular apoptosis. *FASEB J.* **21**, 3250–3261 (2007).
23. Wong, V. W. et al. Focal adhesion kinase links mechanical force to skin fibrosis via inflammatory signaling. *Nat. Med.* **18**, 148–152 (2011).
24. Mascharak, S. et al. Preventing engrailed-1 activation in fibroblasts yields wound regeneration without scarring. *Science* **372**, eaba2374 (2021).
25. Mascharak, S. et al. Multi-omic analysis reveals divergent molecular events in scarring and regenerative wound healing. *Cell Stem Cell* **29**, 315–327 e316 (2022).
26. Clark, J. A., Cheng, J. C. & Leung, K. S. Mechanical properties of normal skin and hypertrophic scars. *Burns* **22**, 443–446 (1996).
27. Confalonieri, P. et al. Regeneration or repair? The role of alveolar epithelial cells in the pathogenesis of idiopathic pulmonary fibrosis (IPF). *Cells* **11**, 2095 (2022).
28. Jandl, K. & Kwapiszewska, G. Stiffness of the extracellular matrix: a regulator of prostaglandins in pulmonary fibrosis? *Am. J. Respir. Cell Mol. Biol.* **63**, 721–722 (2020).
29. Giannandrea, M. & Parks, W. C. Diverse functions of matrix metalloproteinases during fibrosis. *Dis. Model. Mech.* **7**, 193–203 (2014).
30. D'Alessio, S. et al. Revisiting fibrosis in inflammatory bowel disease: the gut thickens. *Nat. Rev. Gastroenterol. Hepatol.* **19**, 169–184 (2022).
31. Rieder, F., Fiocchi, C. & Rogler, G. Mechanisms, management, and treatment of fibrosis in patients with inflammatory bowel diseases. *Gastroenterology* **152**, 340–350 (2017).
32. Yui, S. et al. YAP/TAZ-dependent reprogramming of colonic epithelium links ECM remodeling to tissue regeneration. *Cell Stem Cell* **22**, 35–49 e37 (2018).
33. Moya, I. M. & Halder, G. Hippo-YAP/TAZ signalling in organ regeneration and regenerative medicine. *Nat. Rev. Mol. Cell Biol.* **20**, 211–226 (2019).
34. Lovisa, S. Epithelial-to-mesenchymal transition in fibrosis: concepts and targeting strategies. *Front. Pharmacol.* **12**, 737570 (2021).
35. Liu, L. et al. Epithelial-mesenchymal transition in organ fibrosis development: current understanding and treatment strategies. *Burns Trauma* **10**, tkac011 (2022).
36. Kim, K. K. et al. Epithelial cell alpha3beta1 integrin links beta-catenin and Smad signaling to promote myofibroblast formation and pulmonary fibrosis. *J. Clin. Invest.* **119**, 213–224 (2009).
37. Horejs, C. M. et al. Biologically-active laminin-111 fragment that modulates the epithelial-to-mesenchymal transition in embryonic stem cells. *Proc. Natl Acad. Sci. USA* **111**, 5908–5913 (2014).
38. Tschumperlin, D. J., Ligresti, G., Hilscher, M. B. & Shah, V. H. Mechanosensing and fibrosis. *J. Clin. Invest.* **128**, 74–84 (2018).
39. Wu, M. et al. Identification of cadherin 11 as a mediator of dermal fibrosis and possible role in systemic sclerosis. *Arthritis Rheumatol.* **66**, 1010–1021 (2014).
40. Pittet, P., Lee, K., Kulik, A. J., Meister, J. J. & Hinz, B. Fibrogenic fibroblasts increase intercellular adhesion strength by reinforcing individual OB-cadherin bonds. *J. Cell Sci.* **121**, 877–886 (2008).
41. Urushima, H. et al. Activation of hepatic stellate cells requires dissociation of E-cadherin-containing adherens junctions with hepatocytes. *Am. J. Pathol.* **191**, 438–453 (2021).
42. Mitten, E. K. & Baffy, G. Mechanotransduction in the pathogenesis of non-alcoholic fatty liver disease. *J. Hepatol.* **77**, 1642–1656 (2022).
43. Dasgupta, I. & McCollum, D. Control of cellular responses to mechanical cues through YAP/TAZ regulation. *J. Biol. Chem.* **294**, 17693–17706 (2019).
44. Bracco Gartner, T. C. L. et al. Advanced in vitro modeling to study the paradox of mechanically induced cardiac fibrosis. *Tissue Eng. Part C* **27**, 100–114 (2021).
45. Beyer, C. et al. beta-catenin is a central mediator of pro-fibrotic Wnt signaling in systemic sclerosis. *Ann. Rheum. Dis.* **71**, 761–767 (2012).
46. Watanabe, Y. et al. Integrins induce expression of monocyte chemoattractant protein-1 via focal adhesion kinase in mesangial cells. *Kidney Int.* **64**, 431–440 (2003).
47. Dahl, K. N., Ribeiro, A. J. & Lammerding, J. Nuclear shape, mechanics, and mechanotransduction. *Circ. Res.* **102**, 1307–1318 (2008).
48. Kang, N. Mechanotransduction in liver diseases. *Semin. Liver Dis.* **40**, 84–90 (2020).
49. Duan, J. L. et al. Endothelial Notch activation reshapes the angiocrine of sinusoidal endothelia to aggravate liver fibrosis and blunt regeneration in mice. *Hepatology* **68**, 677–690 (2018).
50. Ding, B. S. et al. Divergent angiocrine signals from vascular niche balance liver regeneration and fibrosis. *Nature* **505**, 97–102 (2014).
51. Dorland, Y. L. & Huveneers, S. Cell–cell junctional mechanotransduction in endothelial remodeling. *Cell Mol. Life Sci.* **74**, 279–292 (2017).
52. Weinbaum, S., Duan, Y., Satlin, L. M., Wang, T. & Weinstein, A. M. Mechanotransduction in the renal tubule. *Am. J. Physiol. Ren. Physiol.* **299**, F1220–1236, (2010).
53. Spasic, M. & Jacobs, C. R. Primary cilia: cell and molecular mechanosensors directing whole tissue function. *Semin. Cell Dev. Biol.* **71**, 42–52 (2017).
54. Chapin, H. C. & Caplan, M. J. The cell biology of polycystic kidney disease. *J. Cell Biol.* **191**, 701–710 (2010).
55. Peyronnet, R. et al. Piezo1-dependent stretch-activated channels are inhibited by Polycystin-2 in renal tubular epithelial cells. *EMBO Rep.* **14**, 1143–1148 (2013).
56. Lee, K. L. et al. The primary cilium functions as a mechanical and calcium signaling nexus. *Cilia* **4**, 7 (2015).
57. Silver, F. H., Siperko, L. M. & Seehra, G. P. Mechanobiology of force transduction in dermal tissue. *Skin. Res. Technol.* **9**, 3–23 (2003).
58. Lumpkin, E. A. & Caterina, M. J. Mechanisms of sensory transduction in the skin. *Nature* **445**, 858–865 (2007).
59. Lee, J. et al. Increased primary cilia in idiopathic pulmonary fibrosis. *Mol. Cell* **41**, 224–233 (2018).
60. Villalobos, E. et al. Fibroblast primary cilia are required for cardiac fibrosis. *Circulation* **139**, 2342–2357 (2019).
61. Shinde, A. V., Humeres, C. & Frangogiannis, N. G. The role of alpha-smooth muscle actin in fibroblast-mediated matrix contraction and remodeling. *Biochim. Biophys. Acta Mol. Basis Dis.* **1863**, 298–309 (2017).
62. Wei, W. et al. Involvement of Smad3 pathway in atrial fibrosis induced by elevated hydrostatic pressure. *J. Cell Physiol.* **233**, 4981–4989 (2018).
63. Russo, I. et al. Protective effects of activated myofibroblasts in the pressure-overloaded myocardium are mediated through smad-dependent activation of a matrix-preserving program. *Circ. Res.* **124**, 1214–1227 (2019).
64. Tanaka, R. et al. Hydrostatic pressure suppresses fibrotic changes via Akt/GSK-3 signaling in human cardiac fibroblasts. *Physiol. Rep.* **6**, e13687 (2018).
65. Okuda, K., Shaffer, K. M. & Ehre, C. Mucins and CFTR: their close relationship. *Int. J. Mol. Sci.* **23**, 10232 (2022).
66. Requena, S. et al. Imaging viscosity of intragranular mucin matrix in cystic fibrosis cells. *Sci. Rep.* **7**, 16761 (2017).
67. Gonzalez-Molina, J. et al. Extracellular fluid viscosity enhances liver cancer cell mechanosensing and migration. *Biomaterials* **177**, 113–124 (2018).
68. Brocker, C., Thompson, D. C. & Vasiliou, V. The role of hyperosmotic stress in inflammation and disease. *Biomol. Concepts* **3**, 345–364 (2012).
69. Miyano, T., Suzuki, A. & Sakamoto, N. Hyperosmotic stress induces epithelial-mesenchymal transition through rearrangements of focal adhesions in tubular epithelial cells. *PLoS ONE* **16**, e0261345 (2021).
70. Narayanan, V. et al. Osmotic gradients in epithelial acini increase mechanical tension across E-cadherin, drive morphogenesis, and maintain homeostasis. *Curr. Biol.* **30**, 624–633 (2020).
71. Mierke, C. T. The matrix environmental and cell mechanical properties regulate cell migration and contribute to the invasive phenotype of cancer cells. *Rep. Prog. Phys.* **82**, 064602 (2019).
72. Moriarty, R. A. & Stroka, K. M. Physical confinement alters sarcoma cell cycle progression and division. *Cell Cycle* **17**, 2360–2373 (2018).
73. Tajik, A. et al. Transcription upregulation via force-induced direct stretching of chromatin. *Nat. Mater.* **15**, 1287–1296 (2016).

74. Bouzid, T. et al. The LINC complex, mechanotransduction, and mesenchymal stem cell function and fate. *J. Biol. Eng.* **13**, 68 (2019).
75. Herum, K. M., Choppe, J., Kumar, A., Engler, A. J. & McCulloch, A. D. Mechanical regulation of cardiac fibroblast profibrotic phenotypes. *Mol. Biol. Cell* **28**, 1871–1882 (2017).
76. Ferrari, S. & Pesce, M. Cell-based mechanosensation, epigenetics, and non-coding RNAs in progression of cardiac fibrosis. *Int. J. Mol. Sci.* **21**, 28 (2019).
77. Elosegui-Artola, A. et al. Force triggers YAP nuclear entry by regulating transport across nuclear pores. *Cell* **171**, 1397–1410 (2017).
78. Lyu, X., Hu, M., Peng, J., Zhang, X. & Sanders, Y. Y. HDAC inhibitors as antifibrotic drugs in cardiac and pulmonary fibrosis. *Ther. Adv. Chronic Dis.* **10**, 2040622319862697 (2019).
79. Alam, S. G. et al. The mammalian LINC complex regulates genome transcriptional responses to substrate rigidity. *Sci. Rep.* **6**, 38063 (2016).
80. Stewart, R. M., Rodriguez, E. C. & King, M. C. Ablation of SUN2-containing LINC complexes drives cardiac hypertrophy without interstitial fibrosis. *Mol. Biol. Cell* **30**, 1664–1675 (2019).
81. Armstrong, J. P. K. et al. A blueprint for translational regenerative medicine. *Sci. Transl. Med.* **12**, eaaz2253 (2020).
82. Seyhan, A. A. Lost in translation: the valley of death across preclinical and clinical divide — identification of problems and overcoming obstacles. *Transl. Med. Commun.* https://doi.org/10.1186/s41231-019-0050-7 (2019).
83. Mascharak, S. et al. Modulating cellular responses to mechanical forces to promote wound regeneration. *Adv. Wound Care* **11**, 479–495 (2022).
84. Friedman, S. L., Sheppard, D., Duffield, J. S. & Violette, S. Therapy for fibrotic diseases: nearing the starting line. *Sci. Transl. Med.* **5**, 167sr161 (2013).
85. Han, J. J. F. D. A. Modernization Act 2.0 allows for alternatives to animal testing. *Artif. Organs* **47**, 449–450 (2023).
86. Swaters, D., van Veen, A., van Meurs, W., Turner, J. E. & Ritskes-Hoitinga, M. A history of regulatory animal testing: what can we learn? *Altern. Lab. Anim.* **50**, 322–329 (2022).
87. Charrier, E. E., Pogoda, K., Wells, R. G. & Janmey, P. A. Control of cell morphology and differentiation by substrates with independently tunable elasticity and viscous dissipation. *Nat. Commun.* **9**, 449 (2018).
88. Cox, C. D., Bavi, N. & Martinac, B. Biophysical principles of ion-channel-mediated mechanosensory transduction. *Cell Rep.* **29**, 1–12 (2019).
89. Parker, M. W. et al. Fibrotic extracellular matrix activates a profibrotic positive feedback loop. *J. Clin. Invest.* **124**, 1622–1635 (2014).
90. Seo, B. R. et al. Collagen microarchitecture mechanically controls myofibroblast differentiation. *Proc. Natl Acad. Sci. USA* **117**, 11387–11398 (2020).
91. Doyle, A. D., Carvajal, N., Jin, A., Matsumoto, K. & Yamada, K. M. Local 3D matrix microenvironment regulates cell migration through spatiotemporal dynamics of contractility-dependent adhesions. *Nat. Commun.* **6**, 8720 (2015).
92. Shellard, A. & Mayor, R. Durotaxis: the hard path from in vitro to in vivo. *Dev. Cell* **56**, 227–239 (2021).
93. Rosales, A. M., Vega, S. L., DelRio, F. W., Burdick, J. A. & Anseth, K. S. Hydrogels with reversible mechanics to probe dynamic cell microenvironments. *Angew. Chem. Int. Ed. Engl.* **56**, 12132–12136 (2017).
94. Caliari, S. R. et al. Stiffening hydrogels for investigating the dynamics of hepatic stellate cell mechanotransduction during myofibroblast activation. *Sci. Rep.* **6**, 21387 (2016).
95. Liu, L. et al. Matrix-transmitted paratensile signaling enables myofibroblast–fibroblast cross talk in fibrosis expansion. *Proc. Natl Acad. Sci. USA* **117**, 10832–10838 (2020).
96. Long, Y., Niu, Y., Liang, K. & Du, Y. Mechanical communication in fibrosis progression. *Trends Cell Biol.* **32**, 70–90 (2022).
97. Liu, L., He, F., Yu, Y. & Wang, Y. Application of FRET biosensors in mechanobiology and mechanopharmacological screening. *Front. Bioeng. Biotechnol.* **8**, 595497 (2020).
98. Kim, T. J. et al. Dynamic visualization of alpha-catenin reveals rapid, reversible conformation switching between tension states. *Curr. Biol.* **25**, 218–224 (2015).
99. Borghi, N. et al. E-cadherin is under constitutive actomyosin-generated tension that is increased at cell–cell contacts upon externally applied stretch. *Proc. Natl Acad. Sci. USA* **109**, 12568–12573 (2012).
100. Pothapragada, S. P., Gupta, P., Mukherjee, S. & Das, T. Matrix mechanics regulates epithelial defence against cancer by tuning dynamic localization of filamin. *Nat. Commun.* **13**, 218 (2022).
101. Baddam, S. R. et al. The desmosomal cadherin desmoglein-2 experiences mechanical tension as demonstrated by a FRET-based tension biosensor expressed in living cells. *Cells* **7**, 66 (2018).
102. Krusche, C. A. et al. Desmoglein 2 mutant mice develop cardiac fibrosis and dilation. *Basic Res. Cardiol.* **106**, 617–633 (2011).
103. Sancho, A., Vandersmissen, I., Craps, S., Luttun, A. & Groll, J. A new strategy to measure intercellular adhesion forces in mature cell–cell contacts. *Sci. Rep.* **7**, 46152 (2017).
104. Morsut, L. et al. Engineering customized cell sensing and response behaviors using synthetic notch receptors. *Cell* **164**, 780–791 (2016).
105. Bugaj, L. J., Choksi, A. T., Mesuda, C. K., Kane, R. S. & Schaffer, D. V. Optogenetic protein clustering and signaling activation in mammalian cells. *Nat. Methods* **10**, 249–252 (2013).
106. Ollech, D. et al. An optochemical tool for light-induced dissociation of adherens junctions to control mechanical coupling between cells. *Nat. Commun.* **11**, 472 (2020).
107. Norona, L. M., Nguyen, D. G., Gerber, D. A., Presnell, S. C. & LeCluyse, E. L. Editor's highlight: Modeling compound-induced fibrogenesis in vitro using three-dimensional bioprinted human liver tissues. *Toxicol. Sci.* **154**, 354–367 (2016).
108. Daly, A. C., Davidson, M. D. & Burdick, J. A. 3D bioprinting of high cell-density heterogeneous tissue models through spheroid fusion within self-healing hydrogels. *Nat. Commun.* **12**, 753 (2021).
109. Kanabekova, P., Kadyrova, A. & Kulsharova, G. Microfluidic organ-on-a-chip devices for liver disease modeling in vitro. *Micromachines* **13**, 428 (2022).
110. Sarkar, U. et al. Metabolite profiling and pharmacokinetic evaluation of hydrocortisone in a perfused three-dimensional human liver bioreactor. *Drug. Metab. Dispos.* **43**, 1091–1099 (2015).
111. Ingber, D. E. Human organs-on-chips for disease modelling, drug development and personalized medicine. *Nat. Rev. Genet.* **23**, 467–491 (2022).
112. Li, W. et al. Matrix stiffness and shear stresses modulate hepatocyte functions in a fibrotic liver sinusoidal model. *Am. J. Physiol. Gastrointest. Liver Physiol.* **320**, G272–G282 (2021).
113. Ford, A. J., Jain, G. & Rajagopalan, P. Designing a fibrotic microenvironment to investigate changes in human liver sinusoidal endothelial cell function. *Acta Biomater.* **24**, 220–227 (2015).
114. Friedrich, C., Endlich, N., Kriz, W. & Endlich, K. Podocytes are sensitive to fluid shear stress in vitro. *Am. J. Physiol. Ren. Physiol.* **291**, F856–865, (2006).
115. Maggiorani, D. et al. Shear stress-induced alteration of epithelial organization in human renal tubular cells. *PLoS ONE* **10**, e0131416 (2015).
116. Miravete, M. et al. Renal tubular fluid shear stress promotes endothelial cell activation. *Biochem. Biophys. Res. Commun.* **407**, 813–817 (2011).
117. Zhou, M. et al. Development of a functional glomerulus at the organ level on a chip to mimic hypertensive nephropathy. *Sci. Rep.* **6**, 31771 (2016).
118. Mastikhina, O. et al. Human cardiac fibrosis-on-a-chip model recapitulates disease hallmarks and can serve as a platform for drug testing. *Biomaterials* **233**, 119741 (2020).
119. Sundarakrishnan, A. et al. Bioengineered in vitro tissue model of fibroblast activation for modeling pulmonary fibrosis. *ACS Biomater. Sci. Eng.* **5**, 2417–2429 (2019).
120. Guenat, O. T. & Berthiaume, F. Incorporating mechanical strain in organs-on-a-chip: lung and skin. *Biomicrofluidics* **12**, 042207 (2018).
121. Sala, F. et al. High-throughput 3D imaging of single cells with light-sheet fluorescence microscopy on chip. *Biomed. Opt. Express* **11**, 4397–4407, (2020).
122. Wijesekara, P. et al. Engineering rotating apical-out airway organoid for assessing respiratory cilia motility. *iScience* **25**, 104730 (2022).
123. Jain, N., Iyer, K. V., Kumar, A. & Shivashankar, G. V. Cell geometric constraints induce modular gene-expression patterns via redistribution of HDAC3 regulated by actomyosin contractility. *Proc. Natl Acad. Sci. USA* **110**, 11349–11354 (2013).
124. Anno, T., Sakamoto, N. & Sato, M. Role of nesprin-1 in nuclear deformation in endothelial cells under static and uniaxial stretching conditions. *Biochem. Biophys. Res. Commun.* **424**, 94–99 (2012).
125. Sakamoto, N., Ogawa, M., Sadamoto, K., Takeuchi, M. & Kataoka, N. Mechanical role of nesprin-1-mediated nucleus-actin filament binding in cyclic stretch-induced fibroblast elongation. *Cell Mol. Bioeng.* **10**, 327–338 (2017).
126. Guilluy, C. et al. Isolated nuclei adapt to force and reveal a mechanotransduction pathway in the nucleus. *Nat. Cell Biol.* **16**, 376–381 (2014).
127. Richeldi, L. & du Bois, R. M. Pirfenidone in idiopathic pulmonary fibrosis: the CAPACITY program. *Expert. Rev. Respir. Med.* **5**, 473–481 (2011).
128. Soare, A. et al. Dipeptidylpeptidase 4 as a marker of activated fibroblasts and a potential target for the treatment of fibrosis in systemic sclerosis. *Arthritis Rheumatol.* **72**, 137–149 (2020).
129. Vorstandlechner, V. et al. The serine proteases dipeptidyl-peptidase 4 and urokinase are key molecules in human and mouse scar formation. *Nat. Commun.* **12**, 6242 (2021).
130. Barry-Hamilton, V. et al. Allosteric inhibition of lysyl oxidase-like-2 impedes the development of a pathologic microenvironment. *Nat. Med.* **16**, 1009–1017 (2010).
131. Munger, J. S. et al. The integrin alpha v beta 6 binds and activates latent TGF beta 1: a mechanism for regulating pulmonary inflammation and fibrosis. *Cell* **96**, 319–328 (1999).
132. Wynn, T. A. & Ramalingam, T. R. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. *Nat. Med.* **18**, 1028–1040 (2012).
133. Chen, K. et al. Disrupting biological sensors of force promotes tissue regeneration in large organisms. *Nat. Commun.* **12**, 5256 (2021).
134. Garoffolo, G. et al. Reduction of cardiac fibrosis by interference with YAP-dependent transactivation. *Circulation Res.* **131**, 239–257 (2022).
135. Peng, X. et al. Inactivation of focal adhesion kinase in cardiomyocytes promotes eccentric cardiac hypertrophy and fibrosis in mice. *J. Clin. Invest.* **116**, 217–227 (2006).
136. Fan, G. P. et al. Pharmacological inhibition of focal adhesion kinase attenuates cardiac fibrosis in mice cardiac fibroblast and post-myocardial-infarction models. *Cell Physiol. Biochem.* **37**, 515–526 (2015).
137. Haak, A. J. et al. Selective YAP/TAZ inhibition in fibroblasts via dopamine receptor D1 agonism reverses fibrosis. *Sci. Transl. Med.* **11**, eaau6296 (2019).
138. Guo, J. L. & Longaker, M. T. Bioprinted hydrogels for fibrosis and wound healing: treatment and modeling. *Gels* https://doi.org/10.3390/gels9010019 (2022).
139. Sun, G. et al. Dextran hydrogel scaffolds enhance angiogenic responses and promote complete skin regeneration during burn wound healing. *Proc. Natl Acad. Sci. USA* **108**, 20976–20981 (2011).
140. Darling, N. J., Sideris, E., Hamada, N., Carmichael, S. T. & Segura, T. Injectable and spatially patterned microporous annealed particle (MAP) hydrogels for tissue repair applications. *Adv. Sci.* **5**, 1801046 (2018).
141. Blackburn, N. J. et al. Timing underpins the benefits associated with injectable collagen biomaterial therapy for the treatment of myocardial infarction. *Biomaterials* **39**, 182–192 (2015).

142. RI, R. I. et al. 3D-printed gelatin methacrylate scaffolds with controlled architecture and stiffness modulate the fibroblast phenotype towards dermal regeneration. *Polymers* **13**, 2510 (2021).
143. Chen, L. et al. 3D-printed dermis-specific extracellular matrix mitigates scar contraction via inducing early angiogenesis and macrophage M2 polarization. *Bioact. Mater.* **10**, 236–246 (2022).
144. Najar-Asl, M. et al. Transplantation of SDF-1α-loaded liver extracellular matrix repopulated with autologous cells attenuated liver fibrosis in a rat model. *EXCLI J.* **21**, 704 (2022).
145. Guan, G. et al. Engineering hiPSC-CM and hiPSC-EC laden 3D nanofibrous splenic hydrogel for improving cardiac function through revascularization and remuscularization in infarcted heart. *Bioact. Mater.* **6**, 4415–4429 (2021).
146. Davidson, M. D., Burdick, J. A. & Wells, R. G. Engineered biomaterial platforms to study fibrosis. *Adv. Healthc. Mater.* **9**, 1901682 (2020).
147. Guo, J. L. et al. Three-dimensional printing of click functionalized, peptide patterned scaffolds for osteochondral tissue engineering. *Bioprinting* **22**, e00136 (2021).
148. Nabai, L., Ghahary, A. & Jackson, J. Localized controlled release of kynurenic acid encapsulated in synthetic polymer reduces implant-induced dermal fibrosis. *Pharmaceutics* **14**, 1546 (2022).
149. Mihalko, E., Huang, K., Sproul, E., Cheng, K. & Brown, A. C. Targeted treatment of ischemic and fibrotic complications of myocardial infarction using a dual-delivery microgel therapeutic. *ACS Nano* **12**, 7826–7837 (2018).
150. Francisco, J. et al. Suppression of myeloid YAP antagonizes adverse cardiac remodeling during pressure overload stress. *J. Mol. Cell Cardiol.* **181**, 1–14 (2023).
151. Xu, W. et al. PCL scaffold combined with rat tail collagen type I to reduce keratocyte differentiation and prevent corneal stroma fibrosis after injury. *Exp. Eye Res.* **217**, 108936 (2022).
152. Horejs, C. M. et al. Preventing tissue fibrosis by local biomaterials interfacing of specific cryptic extracellular matrix information. *Nat. Commun.* **8**, 15509 (2017).
153. Griffin, D. R. et al. Activating an adaptive immune response from a hydrogel scaffold imparts regenerative wound healing. *Nat. Mater.* **20**, 560–569 (2021).
154. Griffin, D. R., Weaver, W. M., Scumpia, P. O., Di Carlo, D. & Segura, T. Accelerated wound healing by injectable microporous gel scaffolds assembled from annealed building blocks. *Nat. Mater.* **14**, 737–744 (2015).
155. Nih, L. R., Sideris, E., Carmichael, S. T. & Segura, T. Injection of microporous annealing particle (MAP) hydrogels in the stroke cavity reduces gliosis and inflammation and promotes NPC migration to the lesion. *Adv. Mater.* https://doi.org/10.1002/adma.201606471 (2017).
156. Jiao, Y. et al. Constructing nanoscale topology on the surface of microfibers inhibits fibroblast fibrosis. *Adv. Fiber Mater.* **4**, 1219–1232 (2022).
157. Sisco, P. N. et al. The effect of gold nanorods on cell-mediated collagen remodeling. *Nano Lett.* **8**, 3409–3412 (2008).
158. Kim, H. et al. Ultrafine carbon black particles inhibit human lung fibroblast-mediated collagen gel contraction. *Am. J. Respir. Cell Mol. Biol.* **28**, 111–121 (2003).
159. Riley, L. A. & Merryman, W. D. Cadherin-11 and cardiac fibrosis: a common target for a common pathology. *Cell Signal.* **78**, 109876 (2021).
160. Trujillo, J. et al. Curcumin prevents cisplatin-induced decrease in the tight and adherens junctions: relation to oxidative stress. *Food Funct.* **7**, 279–293 (2016).
161. Schneider, D. J. et al. Cadherin-11 contributes to pulmonary fibrosis: potential role in TGF-beta production and epithelial to mesenchymal transition. *FASEB J.* **26**, 503–512 (2012).
162. Finch, R. et al. Results of a phase 2 study of RG6125, an anti-cadherin-11 monoclonal antibody, in rheumatoid arthritis patients with an inadequate response to anti-TNFa therapy. *Ann. Rheum. Dis.* https://doi.org/10.1136/annrheumdis-2019-eular.3028 (2019).
163. Beaven, E., Kumar, R., Bhatt, H. N., Esquivel, S. V. & Nurunnabi, M. Myofibroblast specific targeting approaches to improve fibrosis treatment. *Chem. Commun.* **58**, 13556–13571 (2022).
164. Masszi, A. et al. Integrity of cell-cell contacts is a critical regulator of TGF-beta 1-induced epithelial-to-myofibroblast transition: role for beta-catenin. *Am. J. Pathol.* **165**, 1955–1967 (2004).
165. Zhang, J. et al. Adenosine A(2a) receptor blockade diminishes Wnt/beta-catenin signaling in a murine model of bleomycin-induced dermal fibrosis. *Am. J. Pathol.* **187**, 1935–1944 (2017).
166. Tian, Y. et al. A2aR inhibits fibrosis and the EMT process in silicosis by regulating Wnt/beta-catenin pathway. *Ecotoxicol. Env. Saf.* **249**, 114410 (2023).
167. AlQudah, M., Hale, T. M. & Czubryt, M. P. Targeting the renin–angiotensin–aldosterone system in fibrosis. *Matrix Biol.* **91-92**, 92–108 (2020).
168. Ribeiro, M. O., Antunes, E., Muscara, M. N., De Nucci, G. & Zatz, R. Nifedipine prevents renal injury in rats with chronic nitric oxide inhibition. *Hypertension* **26**, 150–155 (1995).
169. Seccia, T. M. et al. Role of angiotensin II, endothelin-1 and L-type calcium channel in the development of glomerular, tubulointerstitial and perivascular fibrosis. *J. Hypertens.* **26**, 2022–2029 (2008).
170. Lin, Y. C. et al. Nifedipine exacerbates lipogenesis in the kidney via KIM-1, CD36, and SREBP upregulation: implications from an animal model for human study. *Int. J. Mol. Sci.* **21**, 4359 (2020).
171. Numaga-Tomita, T. et al. TRPC3-GEF-H1 axis mediates pressure overload-induced cardiac fibrosis. *Sci. Rep.* **6**, 39383 (2016).
172. Connett, G. J. Lumacaftor-ivacaftor in the treatment of cystic fibrosis: design, development and place in therapy. *Drug. Des. Devel. Ther.* **13**, 2405–2412 (2019).
173. Yan, P., Ke, B. & Fang, X. Ion channels as a therapeutic target for renal fibrosis. *Front. Physiol.* **13**, 1019028 (2022).
174. Fan, D. et al. Deciphering the anti-renal fibrosis mechanism of triptolide in diabetic nephropathy by the integrative approach of network pharmacology and experimental verification. *J. Ethnopharmacol.* **316**, 116774 (2023).
175. Raziyeva, K., Kim, Y., Zharkinbekov, Z., Temirkhanova, K. & Saparov, A. Novel therapies for the treatment of cardiac fibrosis following myocardial infarction. *Biomedicines* https://doi.org/10.3390/biomedicines10092178 (2022).
176. Wang, Y. et al. Inhibition of histone deacetylases prevents cardiac remodeling after myocardial infarction by restoring autophagosome processing in cardiac fibroblasts. *Cell Physiol. Biochem.* **49**, 1999–2011 (2018).
177. Barbosa, D. M. et al. Rhein, a novel histone deacetylase (HDAC) inhibitor with antifibrotic potency in human myocardial fibrosis. *Sci. Rep.* **10**, 4888 (2020).
178. Park, K. C. et al. A new histone deacetylase inhibitor improves liver fibrosis in BDL rats through suppression of hepatic stellate cells. *Br. J. Pharmacol.* **171**, 4820–4830 (2014).
179. Xiong, C. et al. Selective inhibition of class IIa histone deacetylases alleviates renal fibrosis. *FASEB J.* **33**, 8249–8262 (2019).
180. Wen, D. et al. Focusing on mechanoregulation axis in fibrosis: sensing, transduction and effecting. *Front. Mol. Biosci.* **9**, 804680 (2022).
181. Saito, S. et al. HDAC8 inhibition ameliorates pulmonary fibrosis. *Am. J. Physiol. Lung Cell Mol. Physiol.* **316**, L175–L186 (2019).
182. Chai, R. J. et al. Disrupting the LINC complex by AAV mediated gene transduction prevents progression of Lamin induced cardiomyopathy. *Nat. Commun.* **12**, 4722 (2021).
183. Chen, X. et al. Suppression of SUN2 by DNA methylation is associated with HSCs activation and hepatic fibrosis. *Cell Death Dis.* **9**, 1021 (2018).
184. Bingham, G. C., Lee, F., Naba, A. & Barker, T. H. Spatial-omics: novel approaches to probe cell heterogeneity and extracellular matrix biology. *Matrix Biol.* **91–92**, 152–166 (2020).
185. Goltsev, Y. et al. Deep profiling of mouse splenic architecture with CODEX multiplexed imaging. *Cell* **174**, 968–981.e915 (2018).
186. Eyres, M. et al. Spatially resolved deconvolution of the fibrotic niche in lung fibrosis. *Cell Rep.* **40**, 111230 (2022).
187. Strunz, M. et al. Alveolar regeneration through a Krt8+ transitional stem cell state that persists in human lung fibrosis. *Nat. Commun.* **11**, 3559 (2020).
188. Wallis, T. J. M. et al. Temporal progression of mediastinal lymphadenopathy in idiopathic pulmonary fibrosis. *Eur. Respir. J.* **59**, 2200024 (2022).
189. Foster, D. S. et al. Multiomic analysis reveals conservation of cancer-associated fibroblast phenotypes across species and tissue of origin. *Cancer Cell* **40**, 1392–1406 (2022).
190. Chen, S., Lake, B. B. & Zhang, K. High-throughput sequencing of the transcriptome and chromatin accessibility in the same cell. *Nat. Biotechnol.* **37**, 1452–1457 (2019).
191. Foster, D. S. et al. Integrated spatial multiomics reveals fibroblast fate during tissue repair. *Proc. Natl Acad. Sci. USA* **118**, e2110025118 (2021).
192. Huang, K. Y. & Petretto, E. Cross-species integration of single-cell RNA-seq resolved alveolar-epithelial transitional states in idiopathic pulmonary fibrosis. *Am. J. Physiol. Lung Cell Mol. Physiol.* **321**, L491–L506 (2021).
193. Fanidis, D., Moulos, P. & Aidinis, V. Fibromine is a multi-omics database and mining tool for target discovery in pulmonary fibrosis. *Sci. Rep.* **11**, 21712 (2021).
194. Wu, Q. et al. Organ-on-a-chip: recent breakthroughs and future prospects. *Biomed. Eng. OnLine* **19**, 9 (2020).
195. Hayward, K. L., Kouthouridis, S. & Zhang, B. Organ-on-a-chip systems for modeling pathological tissue morphogenesis associated with fibrosis and cancer. *ACS Biomater. Sci. Eng.* **7**, 2900–2925 (2021).
196. Bittner, S. M., Guo, J. L. & Mikos, A. G. Spatiotemporal control of growth factors in three-dimensional printed scaffolds. *Bioprinting* **12**, e00032 (2018).
197. Matera, D. L. et al. Microengineered 3D pulmonary interstitial mimetics highlight a critical role for matrix degradation in myofibroblast differentiation. *Sci. Adv.* **6**, eabb5069 (2020).
198. Pakulska, M. M. et al. Encapsulation-free controlled release: electrostatic adsorption eliminates the need for protein encapsulation in PLGA nanoparticles. *Sci. Adv.* **2**, e1600519 (2016).
199. Kumari, J., Wagener, F. & Kouwer, P. H. J. Novel synthetic polymer-based 3D contraction assay: a versatile preclinical research platform for fibrosis. *ACS Appl. Mater. Interfaces* **14**, 19212–19225 (2022).
200. Saleh, K. S. et al. Engineering hybrid-hydrogels comprised of healthy or diseased decellularized extracellular matrix to study pulmonary fibrosis. *Cell Mol. Bioeng.* **15**, 505–519 (2022).
201. Ma, H., Caldwell, A. S., Azagarsamy, M. A., Gonzalez Rodriguez, A. & Anseth, K. S. Bioorthogonal click chemistries enable simultaneous spatial patterning of multiple proteins to probe synergistic protein effects on fibroblast function. *Biomaterials* **255**, 120205 (2020).
202. Georges, P. C. et al. Increased stiffness of the rat liver precedes matrix deposition: implications for fibrosis. *Am. J. Physiol. Gastrointest. Liver Physiol.* **293**, G1147–1154, (2007).
203. Jones, M. G. et al. Nanoscale dysregulation of collagen structure-function disrupts mechano-homeostasis and mediates pulmonary fibrosis. *eLife* **7**, e36354 (2018).
204. Helm, M. et al. Repurposing DPP4 inhibition to improve hair follicle activation and regeneration. *J. Invest. Dermatol.* **143**, 2132–2144 (2023).
205. Ba, Y. D., Sun, J. H. & Zhao, X. X. Evogliptin attenuates bleomycin-induced lung fibrosis via inhibiting TGF-beta/Smad signaling in fibroblast. *Eur. Rev. Med. Pharmacol. Sci.* **24**, 10790–10798 (2020).

206. Kanasaki, K. et al. Linagliptin-mediated DPP-4 inhibition ameliorates kidney fibrosis in streptozotocin-induced diabetic mice by inhibiting endothelial-to-mesenchymal transition in a therapeutic regimen. *Diabetes* **63**, 2120–2131 (2014).
207. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT01963845 (2016).
208. Chen, K. et al. Disrupting mechanotransduction decreases fibrosis and contracture in split-thickness skin grafting. *Sci. Transl. Med.* **14**, eabj9152 (2022).
209. Zhao, X. K. et al. Focal adhesion kinase regulates hepatic stellate cell activation and liver fibrosis. *Sci. Rep.* **7**, 4032 (2017).
210. Lagares, D. et al. Inhibition of focal adhesion kinase prevents experimental lung fibrosis and myofibroblast formation. *Arthritis Rheum.* **64**, 1653–1664 (2012).
211. Jiang, C. et al. Fasudil, a Rho-kinase inhibitor, attenuates bleomycin-induced pulmonary fibrosis in mice. *Int. J. Mol. Sci.* **13**, 8293–8307 (2012).
212. Knipe, R. S., Tager, A. M. & Liao, J. K. The Rho kinases: critical mediators of multiple profibrotic processes and rational targets for new therapies for pulmonary fibrosis. *Pharmacol. Rev.* **67**, 103–117 (2015).
213. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT05832229 (2023).
214. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT04971577 (2023).
215. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT00255242 (2009).
216. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT04385433 (2023).
217. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT01268202 (2019).
218. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT01730339 (2016).
219. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT01037985 (2021).
220. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT01890265 (2020).
221. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT04419558 (2023).
222. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT01217632 (2016).
223. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT01759511 (2017).
224. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT01769196 (2017).
225. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT01672879 (2019).
226. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT01672866 (2019).
227. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT01707472 (2019).
228. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT03949530 (2020).
229. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT04396756 (2023).
230. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT02612051 (2017).
231. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT01371305 (2020).
232. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT00878761 (2020).
233. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT00063869 (2009).
234. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT02453256 (2020).
235. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT04988022 (2023).
236. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT05128383 (2023).
237. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT00987545 (2020).
238. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT01629667 (2017).
239. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT01872689 (2018).
240. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT00786201 (2018).
241. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT02550873 (2022).
242. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT01981850 (2022).
243. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT02305602 (2022).
244. Hussein, K. H., Park, K. M., Yu, L., Kwak, H. H. & Woo, H. M. Decellularized hepatic extracellular matrix hydrogel attenuates hepatic stellate cell activation and liver fibrosis. *Mater. Sci. Eng. C* **116**, 111160 (2020).
245. Chen, K. et al. Pullulan-collagen hydrogel wound dressing promotes dermal remodelling and wound healing compared to commercially available collagen dressings. *Wound Repair. Regen.* **30**, 397–408 (2022).
246. Purcell, B. P. et al. Injectable and bioresponsive hydrogels for on-demand matrix metalloproteinase inhibition. *Nat. Mater.* **13**, 653–661 (2014).
247. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT00958425 (2016).
248. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT02166554 (2014).
249. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT05360186 (2023).
250. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT01226563 (2023).
251. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT01311791 (2017).
252. Landen, N. X., Li, D. & Stahle, M. Transition from inflammation to proliferation: a critical step during wound healing. *Cell Mol. Life Sci.* **73**, 3861–3885 (2016).
253. Gurtner, G. C., Werner, S., Barrandon, Y. & Longaker, M. T. Wound repair and regeneration. *Nature* **453**, 314–321 (2008).
254. Levenson, S. M. et al. The healing of rat skin wounds. *Ann. Surg.* **161**, 293–308 (1965).
255. Gurcan, M. N. et al. Histopathological image analysis: a review. *IEEE Rev. Biomed. Eng.* **2**, 147–171 (2009).
256. Wilder, J. & Patel, K. The clinical utility of FibroScan((R)) as a noninvasive diagnostic test for liver disease. *Med. Devices* **7**, 107–114, (2014).
257. Asilian Bidgoli, A., Rahnamayan, S., Dehkharghanian, T., Grami, A. & Tizhoosh, H. R. Bias reduction in representation of histopathology images using deep feature selection. *Sci. Rep.* **12**, 19994 (2022).
258. Yu, Y. et al. Deep learning enables automated scoring of liver fibrosis stages. *Sci. Rep.* **8**, 16016 (2018).
259. Guo, J. L., Januszyk, M. & Longaker, M. T. Machine learning in tissue engineering. *Tissue Eng. Part A* **29**, 2–19 (2023).
260. Sinha, S. et al. Fibroblast inflammatory priming determines regenerative versus fibrotic skin repair in reindeer. *Cell* **185**, 4717–4736 (2022).
261. Cherry, C. et al. Computational reconstruction of the signalling networks surrounding implanted biomaterials from single-cell transcriptomics. *Nat. Biomed. Eng.* **5**, 1228–1238 (2021).
262. Liu, Y., Bai, F., Tang, Z., Liu, N. & Liu, Q. Integrative transcriptomic, proteomic, and machine learning approach to identifying feature genes of atrial fibrillation using atrial samples from patients with valvular heart disease. *BMC Cardiovasc. Disord.* **21**, 52 (2021).
263. Neuman, K. C. & Nagy, A. Single-molecule force spectroscopy: optical tweezers, magnetic tweezers and atomic force microscopy. *Nat. Methods* **5**, 491–505 (2008).
264. Charras, G. & Yap, A. S. Tensile forces and mechanotransduction at cell–cell junctions. *Curr. Biol.* **28**, R445–R457 (2018).
265. Paluch, E. K. et al. Mechanotransduction: use the force(s). *BMC Biol.* **13**, 47 (2015).

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